DYNAMIC MODIFICATION OF GRAPHITE SURFACES WITH SURFACTANTS FOR ELECTROCHEMICAL DETECTION OF BIOLOGICAL MOLECULES

By

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DYNAMIC MODIFICATION OF GRAPHITE SURFACES WITH SURFACTANTS FOR ELECTROCHEMICAL DETECTION OF BIOLOGICAL MOLECULES

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The purpose of this work was to develop new surfaces for electrochemical analysis of biological molecules that can be applied as detectors for chromatographic methods such as high pressure liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). Electrochemical detectors can be applied to a wide range of compounds and have the advantages of good sensitivity, selectivity and low limits of detection. However electrochemical detection of biological molecules is generally limited by electrode fouling resulting from irreversible adsorption of the analyte at the electrode and irreversible surface changes.

In this work we presented a new strategy developed for the electrochemical detection of small biological surface active molecules and redox proteins at graphite surfaces Graphite surfaces are commonly used as electrochemical detectors in bioanalysis because of properties such as a wide potential window and low background currents The strategy developed for surface modification relied on the use of dynamic surface modification by surfactants at three graphite surfaces -rough pyrolytic graphite (RPG) glassy carbon (GC) and carbon fiber–to reduce problems with surface fouling Surfactants were present in solution during analysis This provided a continuous renewable surface that enhanced the sensitivity of inactive surfaces such as GC and the stability of active surfaces such as RPG

By considering analyte properties such as structure hydrophilicity hydrophobicity and charge surfactants could be chosen to interact favorably with the analyte and with the surface A model for ionic aliphatic surfactant assembly at graphite was developed with small, well characterized catecholamines as electroactive probes, which allowed direct interpretation of the electrochemical data. The proposed model was based on surfactant-surface and surfactant-analyte interactions

Quantitation of response in surfactant media was investigated to determine surfactant-probe interactions and optimum condition in analysis Furthermore, the parameters needed for best response at graphite surfaces such as ionic strength were also investigated

This model was applied to the electrochemical analysis of a redox protein cytochrome c which provided insight into the parameters necessary for good

response of large biological molecules at graphite

CHAPTER 1
INTRODUCTION

1.1 Purpose of Proposed Work

The purpose of this work was to develop new strategies for preparing optimum electrode surfaces for electrochemical analysis of biological molecules that can be applied as detectors for chromatographic methods such as high pressure liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) or in sensors. Electrochemical detectors can be applied to a wide range of compounds and have the advantages of good sensitivity, selectivity, low dead volumes (20μL) and low limits of detections, e.g., 10^{-17} moles for an amperometric electrochemical detector (1). Electrochemical detectors have limits of detection much lower than UV detector with limits of detections between 10^{-15} and 10^{-19} moles (1). Even the sensitive fluorescence detectors with detection limits similar to the electrochemical detectors require tedious sample preparation prior to analysis (1). However, electrochemical detection of biological molecules, especially such large redox proteins as cytochrome c, is generally limited by electrode fouling resulting from irreversible adsorption at the electrode. For example, adsorbed redox proteins typically do not exhibit reversible electron transfer and inhibit the redox reaction of protein from solution, which can eventually result in surface blockage where response can no longer be observed (2) Surface blockage can occur as a result of protein adsorption in native or electroinactive form and as a result of adsorbed surface active trace impurities present in the protein sample In addition to protein adsorption other protein properties such as slow diffusion coefficients and buried redox centers complicate their electrochemical analysis

In this work we present a new strategy developed for the electrochemical detection of small biological molecules as well as redox proteins at graphite surfaces that was aimed at controlling the properties of graphite surfaces relevant in analysis Graphite surfaces are commonly used as electrochemical detectors in bioanalysis because of properties such as wide potential windows (+1 24 V to -1 64 V vs SCE) and low background currents In addition to surface changes caused by irreversible adsorption of biological molecules surface oxidation of graphite can result in the formation of an insulating layer reducing rates of electron transfer at the surface (3) Surface oxidation can increase with increasing solution pH increasing positive potential window of analysis (3) or repeated cycling In addition to external parameters the inherent activity of the surface can determine the susceptibility of the surface to fouling, where an active graphite surface is generally more susceptible to surface oxidation with concomitant surface fouling

In this work graphite surfaces are modified to enhance the sensitivity and reduce problems of surface changes during analysis prolonging the lifetime of the surface while maintaining the inherent properties of graphite useful in bioanalysis. The strategy developed relies on the use of dynamic surface modification by surfactants In this strategy surfactants are present in solution during analysis providing a continuously renewable surface that enhances the stability and allows better control over surfactant assembly at the surface In addition the surface is designed so that electron transfer through this surfactant layer is not significantly hindered Surfactant assembly prevents strong analyte graphite interactions, such as hydrophobic interactions eliminating irreversible adsorption and permanent changes in protein structure in case of protein analysis In addition weak (electrostatic) protein-modified surface and protein-surfactant interactions result in efficient transport of protein to the surface with proper orientation for effective electron transfer. Similar advantages are realized in the analysis of small biological molecules

The results also show how by considering analyte properties such as structure hydrophilicity hydrophobicity and charge surfactants can be chosen to interact favorably with the analyte as well as the surface A model for surfactant assembly at graphite is developed based on the observed surfactant-surface and surfactant-analyte interactions This model was developed with both small and large well characterized biological probes Small biological molecules exhibit fast diffusion and have a more rigid structure less sensitive to environmental parameters allowing straightforward interpretation of electrochemical data. These models were then applied to the development of optimum strategy for electrochemical analysis of proteins and provided insight into the parameters necessary for good response of biological molecules at graphite.

The model for surfactant modification of graphite was developed and applied to conventional structure and size graphite electrodes first. The latter part of this work involved applying this model to an ultramicroelectrode, carbon fiber. Ultramicroelectrodes are commonly used as detectors for CZE because their size facilitates its interface to CZE. In bioanalysis, these electrodes have advantages of lower limits of detection due to a compact double layer resulting in a more efficient mass transfer (electron transfer); lower ohmic potential (iR) drop resulting in their wider applications to solutions of high resistance such as organic solvents and in solutions of low ionic strength, and fast response time making them useful in trace analyses. In addition, their small size makes them useful for in vivo analysis. In particular, carbon fiber is useful in electrochemical detection of biological molecules because of the inherent properties of graphite. Surfactant modification of this surface should improve the response of biological molecules similar to that observed for conventional size electrodes.

1.2 Graphite Surfaces

1.2.1 Conventional Size Electrodes: Glassy Carbon (GC) and Rough Pyrolytic Graphite (RPG)

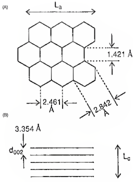
Pyrolytic graphite (PG) is prepared by high temperature (1200°-3800° C) decomposition of gaseous hydrocarbons such as methane and propane onto a hot graphite surface (4). The structural and physical properties of the PG produced depend on the surface of deposition, temperature of heating, and further treatment after manufacturing. PG is roughened to produce RPG using a 600-grit silicon carbide paper.

GC is purchased in the form of disk/rods and is made by thermal degradation of selected organic polymers such as furfural alcohol, phenol formaldehyde and acetone furfural. Precursor materials are cured then carbonized very slowly at temperatures above 300 °C then heated to 1800 °C.

Both GC and RPG are sp^2 carbons with four valence electrons. Three of the four electrons are involved in forming sigma bonds to adjacent carbons with carbon carbon bond length equal to 1.42 Å (5). The fourth electron, is a π electron which is free to move within the graphitic layers.

The properties of graphite are determined by its graphitic orientation. Figure 1 shows basal plane orientation and edge plane orientation of graphite. Basal planes are parallel to the hexagonal layers, while edge planes are perpendicular to these layers. The π electron moves freely within the layers corresponding to edge plane orientation, which is responsible for the greater conductivity observed at edge planes over basal planes. L_a and L_c, shown in Figure 1, correspond to the mean size of graphitic microcrystallite along the axes and the interplanar microcrystallite size respectively. In addition, the interplanar distance d_{002} which corresponds to the stacking of the hexagonal planes (ABAB...) is also shown in this figure. This distance is reported to range between

Figure 1 Crystallographic Dimensions of sp^2 Carbon with (A) view of basal plane, and (B) view of edge plane orientation


(A)
L_a
1.421 Å
2.461 Å
2.842 Å
(B)
3.354 Å
d_002
L_c

between 3.354 and 3.6 Å for different graphite surfaces

PG consists of highly ordered crystallites of carbon in a hexagonal planar array. As shown in Figure 2, highly ordered pyrolytic graphite (HOPG) has well defined graphitic plane orientation with L_a and L_c equal to ca. 500 and 1000 respectively, and d_{002} equal to 3.354 Å. GC is a brittle, hard, amorphous material. This material, shown in Figure 3, consists of trigonally and tetrahedrally bonded carbons in an arrangement of tangled aromatic rings with varying degrees of cross linking. L_a, L_c and L_{002} had been determined to be 50, 15, and 3.359 Å, respectively.

GC and PG have different physical and chemical properties responsible for the observed differences in electrochemical activity of these two surfaces. RPG, which is structurally related to PG, is referred to as an active surface while unactivitated GC is not. The activity of these surfaces is determined by chemical and structural properties such as density of surface functional groups, surface wettability, double layer capacitance, surface roughness and graphitic orientation. Electrochemical activity is characterized by electrochemical measurements of rate constants for electron transfer. The greater activity of RPG than of GC in part is due to a greater amount of exposed edge plane graphite, which is more conductive than basal planes. This leads to a greater density of chemisorbed surface oxides and greater surface wettability.

The graphitic orientation of these surfaces has been determined from polarization sensitive Raman spectra. Raman spectra of highly ordered pyrolytic

Figure 2 Structure of Highly Ordered Pyrolytic Graphite (HOPG)

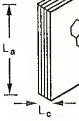
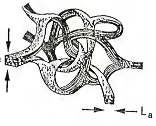

L_a
L_c

Figure 3. Structure of Glassy Carbon (GC)

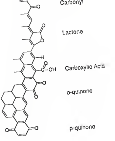

L_c
L_a

graphite (HOPG) have indicate an adsorption wavelength of 1582 cm^{-1} representing basal planes orientation and 1360 cm^{-1} for edge plane orientation (6) The ratio of intensities of adsorption at 1360 cm^{-1} and 1580 cm^{-1} corresponds to the ratio of edge planes to basal plane orientation RPG was found to have a greater ratio of edge planes to basal planes than RPG However this ratio could be increased for GC with pretreatment methods

An example of the commonly chemisorbed surface oxides is shown in Figure 4 Previously it was stated that the amount of chemisorbed surface oxides is greater at the more conductive edge plane orientation Therefore, another measure of surface activity is determined by the amount of surface oxides present at the surface From ESCA analysis the oxygen to carbon (O C) ratios have been determined for PG and GC (7) The results are listed in Table 1 From these results the O C ratio is greater at PG than GC, consistent with the greater activity of this surface

The presence of chemisorbed surface oxides increases the wettability of the surface. In addition to density of surface oxides surface wettability can be related to greater density of edge planes Surface wettability is determined from contact angle studies using the Sessile drop method (6) For a totally wettable surface contact angles of 0° are expected, while for a totally hydrophobic surface contact angles of 90° are observed. The results in Table 1 show RPG has a contact angle of 44 while GC has a contact angle of 66 indicating that RPG is a more wettable surface than GC

Figure 4 Surface Oxides at Graphite Surfaces


OH
O
O
O
H
O
C-OH
O
O
O
O
Phenol
Carbonyl
Lactone
Carboxylic Acid
o-quinone
p-quinone

The double layer capacitance (C_{dl}) of RPG and GC has been determined to be 60 and 12 μF/cm^2 respectively The difference in C_{dl} for GC and RPG is a result of differences in graphitic orientation surface wettability and surface oxides Using C_{dl} measurements and the assumption that the electroactive area is 1 3 times greater than the geometric area (9,10) the roughness factor (RF) of RPG and GC has been determined to be five times greater at RPG as shown in Table 1

In addition to surface properties electrochemical measurements of rate constants for uncomplicated outersphere one electron quasireversible inorganic systems also confirm the differences in activity of RPG and GC The greater activity of RPG over GC is evident from the rate constants for ferricyanide listed in Table 1 which are one order of magnitude greater at RPG than at GC (11)

1 22 Ultramicroelectrodes Carbon Fiber

One of the commonly employed methods in preparing carbon fiber electrodes is catalytic chemical vaporization and deposition (CCVD), which is based on catalytic dehydrogenation of hydrocarbons (e g benzene vapor) on small particles of Fe Ni and Co (4) This method involves diffusion of carbon atoms on the surface or through the bulk of the metal particle after decomposition of the reagent gas The carbon fibers formed can have different structure such as radial, onion and random (Figure 5)

The carbon fiber surfaces have not been extensively characterized Their properties are not as well known as those of other graphite surfaces such as GC

Table 1 GRAPHITE PROPERTIES

SURFACE	OXIDE DENSITY[a] O/C	CONTACT ANGLE[b] (°)	RF[c]	K[d] (10^2 cm s^{-1})
GC[e]	0.12 - 0.17	66	1.3	1.0 ± 0.1
PG[f]	0.24	44	6.5	13 ± 2

[a] Density of surface oxides determined from ESCA analysis (7)

[b] Contact angles measured using Sessile drop method (8)

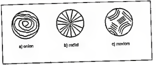
[c] Roughness factor calculated from double layer capacitance measurements assuming microscopic area is 1.3 x geometric area (9,10)

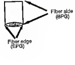
[d] Heterogeneous rate constant for ferricyanide (11)

[e] Garnet alumina polished

[f] Silicon carbide polished (600 grit) to produce RPG

Figure 5 Possible Carbon Fiber Structures (A) radial (B) onion (C) random (D) graphitic plane orientation


a) onion
b) radial
c) random


Fiber side
(BPG)
Fiber edge
(EPG)

and PG. The spectra of carbon fibers prepared at high temperatures are that of highly ordered pyrolytic graphite with preferred graphitic plane orientation (4). The lines in Figure 5 represent edge plane graphitic orientation while the side of the fiber exhibits properties of basal plane graphite. The more active area of the electrode material is located at the edge plane regions (4).

The differences in activities between the disk (edge) and cylindrical (basal) sides of a carbon fiber have been determined from double layer capacitance studies where the disk end had measured capacitance of 23 $\mu F/cm^2$ and the cylindrical side had a capacitance of 6.8 $\mu F/cm^2$. The double layer capacitance measured for pure basal plane orientation is 2-3 $\mu F/cm^2$ indicating that the sides of carbon fiber electrodes do not only have basal planes and that some surface heterogeneity exists (4).

1.2.3 Ionic Strength Effects on Graphite Response

The electrical double layer. The electrical double layer at an electrode surface contains a solution and an electrode side. The solution side is made up of several so-called layers. The inner layer, the layer closest to the surface, contains solvent molecules and possibly other species specifically adsorbed. This layer is referred to as the compact, Helmholtz, or Stern layer. Solvated ions can only approach the surface to the outer part of the Helmholtz layer known as the outer Helmholtz layer. The interactions of the solvated ions with the charged surface involve only long-range electrostatic interactions. Therefore, these interactions are independent of chemical properties of the analyte and these analytes are nonspecifically adsorbed. On the other hand, specifically adsorbed species can approach the surface as close as the inner Helmholtz layer and interactions are dependent of chemical properties of the analyte.

Electrode kinetics are affected by the double (Helmholtz) layer structure by adsorption of reactants, products, supporting electrolytes, along with any other species in solution. As the concentration of the supporting electrolyte is increased, the double layer becomes more compact, allowing better surface access for the electroactive probe (12), which can result in an increase in rates of electrode reactions measured by increase in current. On the other hand, decreasing concentration of the supporting electrolyte produces a less compact (diffuse) double layer, extending the outer Helmholtz region further into solution and preventing solvated ions to access the surface closely. The electrolyte composition can determine the size and properties of the double layer, which in turn can affect rates of electrode reactions according to the classical Frumkin analysis of double layer effects on kinetics (13).

The effect of double layer composition on analyte-surface interactions will depend on the nature of these interactions. For instance a specifically adsorbed analyte can approach the surface as closely as the inner Helmholtz layer; changes in the double layer size, which changes the location of the outer Helmholtz layer, can significantly affect nonspecifically adsorbed analytes which can only access the surface as closely as the outer Helmholtz layer, but not analytes which are specifically adsorbed. However, in the case where analytes are specifically adsorbed at charged electrode sites, changes in the double layer composition could alter the availability of these charged surface sites.

Double layer effects on the electrochemical response of graphite. In the case of graphite, chemisorbed functional oxide groups produce a negatively charged surface at neutral pH and moderate potentials. Specific adsorption or preferential interactions with cations have been reported (14-16). Since specifically adsorbed cations on graphite result from electrostatic interactions with the surface, it can be expected that surface charge screening by the supporting electrolyte cations can improve response for anionic species and reduce that for cationic ones by changing the availability of these sites. This can occur by increasing ionic strength and/or adding a supporting electrolyte with multiply charged cations (13). In addition to electrostatic interactions, hydrophobic interactions at graphite can occur. These interactions are dependent on the extent of exposed hydrophobic groups and the ability of the hydrophobic moiety of the analyte to access these regions. Access to these regions should be optimal when the double layer is compact and the outer Helmholtz layer is closest to the surface.

Charge screening effects were shown by Hill and Whitford (17) to be important to the response of 4-chloro-3,5-dinitrobenzoic acid (CDNB) substituted cytochrome c (net negative charge) at pyrolytic graphite (PG). The results demonstrated an improvement in response with increased ionic strength or adding a supporting electrolyte with multivalent cations. The increase in response was attributed to effective screening of the anionic surface groups allowing the CDNB-substituted cytochrome c to access the surface efficiently producing higher rates of electron transfer

Recent work by Anson and Lee (18) on carbon microdisk electrodes demonstrated an improvement in response of negatively charged ferricyanide following addition of a supporting electrolyte with multivalent cations This improvement was attributed to screening of the surface functional groups by the cations along with increased transport of the ferricyanide through the double layer, as a result of a decrease in net charge of the ferricyanide-cation complex.

1 3 Redox Proteins

1 31 Structure and Analysis

Electrochemical detection of redox proteins is limited because of their adsorption at the surface resulting in surface blockage and eventual loss of signal Biological molecules such as redox proteins have complicated structures consisting of four parts the primary structure which is the amino acid sequence the secondary structure which is the orientation of this amino acid sequence such as α helix or β sheet, the tertiary structure which, is the three dimensional protein structure and the quaternary structure (not observed for all proteins), which consists of two or more protein subunits as in hemoglobin

Changes in tertiary structures can occur easily because of the sensitivity of protein structure to environmental changes such as temperature and pH Because proteins consist of hydrophobic and hydrophilic regions, they can alter their structure to interact with the surface or its environment resulting in irreversible adsorption at most surfaces This adsorption is usually a result of permanent structural changes in the protein and complicates electrochemical analysis of proteins in two ways First the electroactive area is reduced, since irreversible protein adsorption blocks the surface Second the existence of multiple protein forms prevents chemical identification of the protein

1 32 Protein Electrode Surface Interactions

Previous electrochemical studies of redox proteins such as cytochrome c (cyt c) at different electrode surfaces have enabled a model for protein-electrode surface interactions to emerge (2) Hydrophilic proteins such as cyt c (outer hydrophilic surface) with hydrophobic cores usually expose their hydrophobic interior at surfaces with hydrophobic regions resulting in permanent protein conformational changes and loss of bioactivity The process of electrostatic attraction bringing protein close to the surface and leading to exposure of its hydrophobic interior is known as cooperative interaction and is unwanted in analysis This process has been observed by several groups at various graphite surfaces For example Hill and colleagues (19 20) conducted studies of cyt c on graphite surfaces concluding that interactions between the protein and graphite occur mainly due to electrostatic attraction between the negative surface oxide groups present at graphite (at pH 5 6 or greater) and the positive (lysine) groups around the prosthetic (heme) group of cyt c (isoelectric point of 10 0) These interactions resemble electrostatic interactions of cyt c in vivo that are reported to occur between the lysine groups on cyt c and the carboxylate groups of its redox partner cyt c oxidase and phosphate groups on phospholipid membranes (21). Studies of cyt c response at basal plane pyrolytic graphite (BPG) with low density of surface oxides and edge plane pyrolytic graphite (EPG) with a greater amount of surface oxides indicated the response of cyt c to be better at EPG than BPG. In addition, at the more hydrophobic surface, BPG surface fouling was a greater problem (19,20). Surface fouling was also observed by Bond et al at GC where a decrease in cyt c response was observed with increasing scan number due to the presence of the irreversibly adsorbed protein form at the surface (22).

In addition to hydrophobic interactions, other protein-surface interactions can lead to permanent changes in protein structure, such as the formation of a chemical bond between the protein and the surface. For example, extensive studies of cyt c response on gold (Au) indicated that a covalent bond forms between a sulfur group of an amino acid methionine on cyt c and the Au surface, resulting in irreversible protein adsorption with a concomitant conformational change of cyt c (23). Conformational change has been reported to be a result of a bond breakage between the prosthetic heme group iron and sulfur of cyt c, with a change in coordination number of the iron from six to five (24).

From these results it can be postulated that an ideal surface for electroanalysis of cyt c and other redox proteins is one at which the protein will not interact irreversibly. This entails using a surface that matches the properties of the exterior of the protein without inducing protein unfolding. In other words, for hydrophilic proteins with charged groups on its exterior, the ideal surface is one that could electrostatically or hydrogen bond with the exterior groups on the protein but could not interact with its interior. In the case of cyt c, weak electrostatic interactions between cyt c and the surface may be tolerated but not hydrophobic ones because these can result in unfolding of cyt c. Tin oxide has been thought to be an ideal surface for cyt c analysis and has been extensively used by Bowden et al. (25) to investigate the effects of parameters such as ionic strength on adsorption of cyt c. This surface is limited in bioanalysis due to a narrow potential window and sensitivity to alkaline pH, where the surface undergoes degradation at highly alkaline pH (26). The potential window of tin oxide in aqueous media lies between 0 and 2 V vs Ag/AgCl. It is limited in the negative potential region due to hydrogen discharge and concurrent reduction of tin oxide to metallic tin with concomitant surface destruction (26).

1.4 Strategies in Surface Modification for Analysis

1.4.1 Polymer-Modified Electrodes

Polymer modified electrodes (PME) are formed by attachment of a polymer layer at the electrode surface. Attachment of polymers to the surface can be done by various methods (27-31). Of particular interest to our work are PMEs formed with ion exchanged polymers. Modification here consists of attaching a polymer film of positive or negative charged polyelectrolytes developed to electrostatically trap oppositely charged species (32-35). The electrochemical response observed at these polymer-modified surfaces depends on the ability of analytes to partition into the film and diffuse through the adsorbed polymer film. These films tend to partition ions following normal ion exchange patterns (33). Selectivity of these films arises from the charge and chemical differences between the analytes (34).

The most studied ion exchange polymer films are perfluorosulfonate Teflon such as Nafion®. This polymer consists of hydrophilic regions from the sulfonate groups (SO$_3^-$) and hydrophobic region as a result of the surrounding hydrophobic polymer (35). Nafion PMEs are, therefore, cation exchangers that exclude anions with preferential partitioning of hydrophobic over hydrophilic species (36-38).

Unfortunately, these surfaces have numerous drawbacks in analysis. Since response at these PMEs is governed by physical partitioning of probes from solution into the film, the voltammetric response is due to preconcentration of probes in the film. This requires time. Response times vary depending on the nature and size of the probe. Response times of minutes (39) have been observed for small probes, while response times of several days (40) have been observed for large hydrophobic probes. In addition, because response is dependent on partitioning, these films suffer from saturation of the electrostatic sites (39-41) and interference due to competition for these sites by other ions in solution (42). Other problems such as control of film thickness, analyte properties, required sensitivity, and matrix effects, pose problems in the design and use of PMEs Usually some knowledge of sample composition and concentration is required for the effective use of PMEs

1 42 Self Assembly and Langmuir Blodgett Methods in Electrode Modification

Self assembled monolayers (SAM) have been proposed as electrode modifiers for improvement in sensitivity selectivity, and reducing memory effects Modification of surfaces by SAM is done by adsorbing a modifier from a solution prior to conducting analysis at the surface This is generally a combination of two processes binding of a functional group of the reagent to the surface with concomitant hydrophobic and van der Waals attraction between hydrophobic segments of the reagent (43)

Similar to SAM Langmuir Blodgett (LB) films have been used to manipulate electrode response Under LB methods an amphophilic modifier is spread at the air/solvent interface and pressure is applied at this interface The substrate (surface) is dipped into solution and pressure is applied to compress molecules to form a film at the surface Pressure is maintained while the substrate is inserted and removed from the solution Control of coverage is determined by how often the substrate is inserted into the modifier solution which can result in monolayer or multilayer coverage Control of response sensitivity and selectivity is done by varying the modifier the surface or the solvent since orientation of the modifier at the surface depends on the nature of the surface modifier and solvent used LB films are usually compact although they are reported to relax once exposed to analyte solution

The modifiers used in SAM and LB methods normally have both hydrophilic and hydrophobic properties. For example, in particular interest to our work are alkanethiols, which are commonly used as gold (Au) modifiers through SAM. Interest in these alkanethiol modifiers arose from their well defined microstructure, which allows investigating relationships between molecular microstructure at the electrode surface and macroscopic electrochemical observations such as sensitivity. The microstructure of self-assembled monolayers has provided insight into electron-transfer reactions at electrodes (44,45).

Alkanethiol modifiers are adsorbed at Au by forming a covalent bond through a sulphur group onto the Au surface (24,25,46-48). Another group present in the modifier is left facing the solution, capable of interacting with the analyte through weaker interactions, usually hydrogen bonding or electrostatic attraction. The group facing solution and the one adsorbed at the surface are linked usually by a hydrophobic group (long aliphatic or bulky), giving the modifier a far more rigid structure. The stability of the monolayer depends on the length of this hydrophobic region, where hydrocarbon chains of 10 carbons or more have been reported to form the most stable films.

These modifiers have proven successful in the analysis of proteins such as cyt on Au. In the absence of modifiers, cyt c adsorbs irreversibly on Au, leading to eventual loss of signal due to partial surface blockage. In the presence of the alkanethiols, direct interactions between cyt c and Au are prevented. In addition a good response for cyt c is observed due to favorable interactions between cyt c and the modifier. Both SAM and LB methods provide permanently modified electrodes which can improve the sensitivity and selectivity of response as well as reduce memory effects at the surface.

Response at these films is determined by film thickness, compactness and the ability of probes to partition into these films, which is governed by the compactness of the film and the properties of the analyte. Interactions between modifiers and analyte usually are a result of electrostatic attractions, hydrogen bonding, and hydrophobic partitioning. Since modifiers with 10 or more carbon chain length are required for formation of a stable film at the surface, a significant increase in distance between the probe and surface is encountered with modification. This results in decreases in electron transfer for probes at compact layers or for probes that cannot penetrate deep into the SAM or LB film because of their size or structure limiting the use of these surfaces especially for sensitive amperometric analysis.

1.4.3 Surfactant Applications in Surface Modification

Surfactant modification of graphite was shown to be useful in analysis of biological molecules as a result of the similarities between surfactant assemblies and biological membranes. The desired membrane properties such as charge, hydrophobicity and assembly structure can be controlled by the choice of surface, surfactant, and solution conditions.

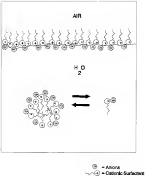
Aliphatic surfactants. Of particular interest to our work are ionic aliphatic surfactants These surfactants consist of a hydrophobic tail consisting of an alkyl chain and a charged hydrophilic head group such as quaternary ammonium salts, sulfates and sulfonates An example of assembly of ionic aliphatic surfactants at an air/H_2O interface is shown in Figure 6. At this interface aliphatic surfactants align perpendicular to the interface with the hydrophilic head group in solution and the hydrophobic tail group facing the air

In bulk aqueous media as a result of hydrophobic interactions between neighboring surfactant tail groups surfactants form normal micelles in solution at a certain concentration known as the critical micelle concentration (cmc) At and above cmc a constant concentration of monomer surfactants exist in solution These monomer surfactants are in continuous exchange with surfactants in micelles

Reported cmc values for aliphatic surfactants are usually in the millimolar range Since micellation is a result of hydrophobic repulsion between the hydrophobic surfactant tail groups and water surfactants which are more hydrophobic (e g longer tail group) will have lower cmc than more hydrophilic ones. In addition cmc of aliphatic surfactants is affected by external solution parameters such as ionic strength and nature of the supporting electrolyte For example as the electrolyte concentration is raised repulsion between neighboring head groups is reduced resulting in a decrease in cmc

Normal micelles are micelles formed in water As shown in Figure 6, they are spherical like vesicles with the hydrophilic head groups facing solution and

Figure 6 Surfactant assembly at an Air/H_2O Interface and Micellation in Aqueous Media. This is a cartoon representation for visual purposes only


AIR
H O
2
= Anions
= Cationic Surfactant

the hydrophobic tails making up the interior core Their diameter ranges between 20-30 Å (49-51) depending on the carbon chain length as well as the nature (size/charge) of the hydrophilic head group The diameter of the micelle is determined by the length of the carbon chain length Increasing carbon chain length increases micelle size In addition the number of surfactants per micelle or the aggregation number determines micelle size Surfactants with lower charged head groups generally have the larger aggregation numbers due to weaker head group repulsion between neighboring surfactants

Aggregation number similar to cmc, is affected by external parameters such as electrolyte composition Aggregation numbers increase with ionic strength or addition of electrolytes with multiply charged ions This is a result of a decrease in head group repulsion between neighboring surfactants For aliphatic surfactants aggregation numbers between 60 and 60 have been reported (49-51)

The interactions of surfactants with species present in solution are well documented (49 51) Prior to micelle formation ion pairing interactions between analytes and aliphatic (ionic) monomer surfactants occur After the formation of micelles the solubility of otherwise insoluble hydrophobic molecules in water increases The ability of micelles to solubilize species otherwise insoluble in water is advantageous in biological analysis This solubilization process occurs through reversible (dynamic) interactions between the analyte and micelles in solution Solubilized species may reside in various regions of a micelle (49-51)

Some penetrate into the hydrophobic core, some remain near the micelle solution interface around the hydrophilic head groups, and others are located somewhere between these two regions. the extent of solubilization in a micelle depends on the hydrophobicity of the analyte. A more hydrophobic analyte will partition deeper into the micelle core than a hydrophilic analyte which will remain primarily at the surface and perhaps electrostatically interact with the micelle. Solubilization properties of micelles can be controlled by using surfactants with longer tail groups which produce larger more hydrophobic micelles.

Surfactants are amphophilic molecules with hydrophobic and hydrophilic properties. A characteristic feature of surfactants is their tendency to adsorb at a solid-liquid interface (51,52). Surfactant adsorption at surfaces in aqueous media could be a result of ion exchange, ion pairing, hydrogen bonding (Lewis acid Lewis base reactions), π-π interactions, hydrophobic interactions, and by London-van der Waals dispersion forces (51).

At hydrophilic surfaces in aqueous media assembly of aliphatic surfactants is thought to occur through its head groups. Lateral interactions between neighboring surfactant at the surface result in formation of bilayer-like assemblies at the surface (51,52). If surfactant concentration is low resulting in low surface coverage this assembly is loose with significant amount of defects while as surface coverage is increased the assembly becomes more compact and ordered. At hydrophobic surfaces, tail groupe interactions with the surface drive assembly and only monolayers in aqueous media are expected.

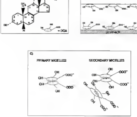
Bile surfactants Bile surfactants are biological surfactants produced in the liver and used in the intestines to dissolve ingested fat to allow its adsorption by the intestinal wall (53) Unlike aliphatic surfactants bile surfactants have no clear cut polarities where regions of hydrophobicities and hydrophilicities are not as well defined as in aliphatic surfactant

Bile surfactants have a bulky structure with no defined head or tail group (53) The bean shape structure of bile surfactants in particular deoxycholic acid (DCA) is shown in Figure 7 Bile surfactants consist of a rigid steroid nucleus with a hydrophilic and hydrophobic side The hydrophilic side of bile surfactants has 2-3 hydroxyl groups and a carboxylic group which is ionized at pH ≥ 8 (pKa = 6 8) The hydrophobic side of bile surfactants consists of 2 methyl groups located at the 18β and 19β position.

Naturally occurring biles vary in the number of hydroxyl groups as well as the isomeric orientation of these groups Human bile surfactants are conjugated with the amino acids glycine and taurine through a covalent bond between the amine groups of the amino acids and the carboxylic groups of bile surfactants similarly to peptide bonds formed between two amino acids This results in lower pKa s of bile of ca 4 and 2 respectively which prevents precipitation of bile at physiological pH

Bile surfactants aggregate in aqueous solution initially as a result of back to back (hydrophobic) interactions between neighboring surfactants (53 55) with the amino acids glycine and taurine through a covalent bond between the

Figure 7 Structure and Properties of Bile Surfactants (A) deoxycholic acid structure (DCA); (B) assembly at an air/H_2O interface and at surfaces in aqueous media; (C) aggregation in aqueous solution


A)
= DCA
B)
AIR
H_2O
SURFACE
C)
PRIMARY MICELLES
SECONDARY MICELLES
OH
COO⁻

amine groups of the amino acids and the carboxylic groups of bile surfactants similarly to peptide bonds formed between two amino acids This results in lower pKa s of bile of ca. 4 and 2 respectively which prevents precipitation of bile at physiological pH

Bile surfactants aggregate in aqueous solution initially as a result of back to back (hydrophobic) interactions between neighboring surfactants (53-55) These hydrophobic interactions result in the formation of primary micelles as shown in Figure 7 with aggregation numbers less than 10 Through H bonding interactions primary micelles are reported to form secondary micelles also shown in Figure 2C resulting in larger aggregates (55)

Although aggregation begins at the critical micelle concentration (cmc) even below this concentration dimers and trimers are reported Beyond cmc unlike for aliphatic surfactants, bile concentration continues to increase with increasing bile concentrations (53-55)

Figure 7 shows assembly of bile at the air/H_2O interface Assembly of bile at air/water (acidic aqueous solutions) under Langmuir Blodgett methods has been studied by Ekwall et al (56,57) Orientation of bile at these interfaces is thought to occur with the methyl groups facing the air interface and the OH and COOH groups facing the aqueous substrate It was found that for bile surfactants with two hydroxyl groups such as lithocholic and glycolithocholic acids assembly was flat where the longitudinal axis of bile is parallel to the surface so that both hydroxyl groups are in contact with the aqueous substrate

Even when pressure was applied compressing the carboxyl and hydroxyl groups these groups still remained in contact with the aqueous substrate and flat at the surface because of the positioning of the OH groups Similarly, because of the positioning of the OH groups of other bile surfactants such as dihydroxy and trihydroxy biles their assembly is parallel to the surface On the other hand, bile surfactants with one hydroxyl group are reported to assemble perpendicular to the aqueous substrate (56) Ekwall and Ekholm et al observed that monolayer formation at the surface occurred easily because of the amphophilic properties of bile surfactants (56 57) However, multilayer formation required large amounts of energy For example 3000 cal/mol of energy was required for the formation of a termolecular structure at the surface and bilayer structures were not observed

Surfactant effect on electrochemical response The use of surfactants in electrochemistry has been documented in various reviews (49-51 58,59) The concentration and nature of the surfactants can affect electrochemical response by changing the shape of electrochemical wave the half wave potential diffusion coefficients peak currents electron transfer rates and the stability of intermediate species (49 50 60 67) The cited studies discussed the effect of solution interactions in micellar media on probe response Work by Aouyagui et al (68) has shown the feasibility of calculating standard formal potentials $E^{\circ\prime}$ of water insoluble substances by using electrochemical measurements in micellar aqueous media in conjunction with solubility measurements Electrochemical response will be affected by the strength of micelle-probe interactions (68,69) the structure and size of the micelle (69-71) which was shown earlier to be influenced by supporting electrolyte as well as the degree of partitioning of the probe into the micelle (49,64,68) However, when considering micellar effect on electrochemical response both surface-micelle interactions and probe micelle interactions must be considered

Previous work by Bard and coworkers (52) on the reductive electrochemistry of methyl viologen in micellar solutions of anionic sodium dodecyl sulfate (SDS) showed that the hydrophobic cation radical intermediate of methyl viologen (MV^{+}) partitioned deeper into the micelles of anionic SDS than the more hydrophilic forms of methyl viologen such as MV and MV^{2+} This greater partitioning resulted in positive shifts in half-wave potentials ($E_{1/2}$) consistent with increased difficulties in oxidation (electron transfer) In addition despite the more favorable electrostatic interactions between MV^{2+} and SDS than of MV^{+} and SDS kinetics for the more hydrophilic form, MV^{2+} were not significantly changed in micellar SDS solution Their results indicated that the electrochemical response of hydrophobic probes would be more affected than the response of hydrophilic probes as a result of deeper partitioning into the micelle core making it more difficult for electron transfer with the electrode to occur

In another study, Bard and coworkers (72) used cyclic voltammetry (CV) measurements to investigate structure and electron transfer properties of surfactant aggregates with electroactive head groups formed under Langmuir-Blodgett methods They observed increase in peak currents when surfactant multilayers were adsorbed tail-head tail-head on tin oxide The response was attributed to the instability of the surfactant multilayers Unfavorable surfactant surfactant and surfactant surface interactions resulted in defects in the multilayers resulting in better electrode access where electron transfer was facilitated at these defects However, when surfactants were adsorbed head-tail-tail-head at tin oxide peak currents decreased as a result of greater electrode hindrance Surfactant bilayers were more stable when surfactants adsorbed head on and interacted hydrophobically with hydrocarbon tail groups of a second layer Electrode hindrance at these bilayers resulted from the formation of more compact assembly at the surface where electron transfer distance was increased They also observed a decrease in rate of electron transfer following an increase in surfactant layer thickness where electron transfer was still possible but sluggish at a multilayer coverage

In related work Pouillen et al (73) reported negative shifts in reduction potential for nitrobenzene in anionic (SDS) and nonionic (Brij-35) surfactants at GC and a decrease in currents in micellar media which they attributed to an increased difficulty of electron transfer through an adsorbed surfactant layer However in positive (CTAB) surfactant solutions response for nitrobenzene was improved They concluded that CTAB micelles decreased protonation rate of the nitrobenzene anion radical and facilitated reduction by a dianion pathway The negative and nonionic surfactants increased the stability of the anion radical and slowed down the rate of radical anion reduction.

Mousty and Mousset (74) saw a decrease in electron transfer for 4-acetyl-1-phenoxyalkyl ammonium salts, electroreducible amphophilic molecules of short carbon chain length in CTAB or Brij-35 solutions. They attributed this to an increase in difficulty of reduction through the adsorbed surfactant layer. However, as the carbon chain length was increased for the electroactive amphophiles, the kinetics improved. This was attributed to the formation of mixed micelles between the non-electroactive surfactants and the electroreducilbe ones which resulted in an adsorbed mixed micelle layer at the surface at which reduction of the electroactive amphophiles was facilitated.

Ewing and Wallinford reported decrease in currents for catecholamines at carbon fiber electrodes which were used as detectors for CZE analysis in micellar media of SDS at cmc (10 mM) (75). They attributed this decrease in response to the partitioning of the probes into the interior of the micelles which resulted in a decrease in diffusion coefficient.

However, work conducted by this group using aliphatic surfactants in the analysis of catecholamines at RPG and GC showed enhancements in response at inactive GC and a reduction of surface fouling at RPG (76). The slight decrease in response observed at RPG was consistent with the decrease in probe adsorption at the surface and did not appear to be a result of solubilization of probe in micellar media which would result in an apparent decrease in the diffusion coefficient This work indicated that in order for surfactants to improve response at graphite favorable probe-surfactant, and surfactant-surface interactions must occur

Quantitation of electrochemical response in surfactant media. Several methods for quantitating electrochemical response for analyte in micellar media have been reported (49,66) Micelle-analyte equilibrium association constants e g , binding constants have been determined using electrochemical methods These methods involve comparing half-wave potential ($E_{1/2}$) in micellar media to the $E_{1/2}$ measured in water Shifts in $E_{1/2}$ can be used to determine the extent of probe micelle interactions (49 66) Ohsawa and Aoyagui developed a mathematical relationship between $E_{1/2}$ and the binding constants of micelles with analyte (49) The derivation of this equation is shown below $E_{1/2}$ for a reversible system determined from CV experiments is

$$E_{1/2} = \frac{E_{pa} + E_{pc}}{2} \qquad (1)$$

where E_{pc} and E_{pa} are cathodic and anodic peak potentials (mV), respectively At 25 °C $E_{1/2}$ can be related to the thermodynamic formal potential (E°') by

$$E_{1/2} = E^{o\prime} + \frac{0.05916}{n} \log \left(\frac{D_r}{D_o}\right)^{1/2} \qquad (2)$$

where n is the number of electrons transferred per mole and D_r and D_o are the diffusion coefficients (cm^2/s) for the reduced and oxidized forms of the redox couple respectively (12). For a system:

$$\begin{array}{ccc} Ox_{(aq)} + e & \rightleftharpoons & Red_{(aq)} \\ Micelles \uparrow\downarrow K_o & & K_r \uparrow\downarrow Micelles \\ Ox_{(M)} + e & \rightleftharpoons & Red_{(M)} \end{array} \tag{3}$$

where the subscripts aq and M represent analyte in the aqueous and micellar media, respectively, the equilibrium binding constants for the reduced (K_r) and the oxidized form (K_o) are

$$K_r = \frac{[Red_{(aq)}]}{[Red_{(M)}]\ [Micelles]} \tag{4}$$

and

$$K_o = \frac{[Ox_{(aq)}]}{[Ox_{(M)}]\ [Micelles]} \tag{5}$$

The Nernst equations at 25°C for equations 1 and 2 above are

$$E_{aq} = E^{o'}_{(aq)} + \frac{0.05916}{n} \log \frac{[Ox_{(aq)}]}{[Red_{(aq)}]} \tag{6}$$

and

$$E_M = E^{o'}{}_{(M)} + \frac{0.05916}{n} \log \frac{[Ox_{(M)}]}{[Red_{(M)}]} \qquad (7)$$

Substituting equations 4 and 5 into equation 7 gives

$$E_M = E^{o'}{}_{(M)} + \frac{0.05916}{n} \log \frac{K_r\,[Ox_{(aq)}]}{K_o\,[Red_{(aq)}]} \qquad (8)$$

At 25°C $E_{1/2} = E^{o'}$, if [Ox] = [Red], using equation 8 shifts in $E^{o'}$ ($\Delta E^{o'}$) can be determined from the equation below

$$\Delta E^{o'} = E^{o'}{}_{(aq)} - E^{o'}{}_{(M)} = \frac{0.05916}{n} \log \left(\frac{K_o}{K_r}\right) \qquad (9)$$

For a reversible system $E^{o'} = E_{1/2}$. $E^{o'}$ measured in micellar solutions can be compared to $E^{o'}$ in aqueous media. These shifts in $E^{o'}$ can be used to determine the binding constant ratios of the oxidized and the reduced forms of the probe to micelles K_o/K_r which is a measure of a degree of probe micelle binding. For example from equation 9 shift in $E_{1/2}$ indicates that the binding of the oxidized form of the analyte to micelles is greater than the reduce form since K_o is greater than K_r.

Using this method and the above equation 9 the binding constants for catecholamines in aliphatic micellar media have been previously calculated (76). Similar methods will be employed in this work to investigate the binding constant ratios for proteins to surfaces and surfactants

1 5 Application of Amphophile Modified Graphite Surfaces in Bioanalysis

Modification of graphite for biological analysis has been attempted previously by exploiting surface active amphiphilic modifiers Patriarche et al (77) modified GC by depositing a hydrophobic coating of phospholipids consisting of different amounts of phosphate groups (negatively charged at neutral pH) to selectively detect positively charged analytes with hydrophobic moieties Tetracaine and chlorpromazine which are positively charged (contain tertiary nitrogen) and are highly soluble in lipids (enables them to partition into the adsorbed phospholipid layer) were detected with better selectivity than negatively charged species such as ascorbic acid Similarly, Kader et al (78) modified GC with phosphytidylcholine to show the selectivity of the surface attached membrane-like structure for hydrophobic ferrocene derivatives containing positively charged side groups

Other work conducted by O'Neill and Lyne (79) involved using stearic acid which is negatively charged at pH 7 to modify carbon paste electrode This allowed electroanalysis of DA without AA interference The carbon paste electrode was produced by mixing graphite powder and stearic acid in Nujol oil This mixture was then packed into a Teflon coated silver wire by pressing the paste into a well of the electrode body about 2 cm high Their results indicated increase in probe distance to the surface following modification and a significant decrease in response of negatively charged AA but not positively charged DA.

Matolla and Albahadily (80) used 0 10% (w/v) two neutral and one anionic surfactant Brij-35 Triton x, and sodium dodecyl benzene sulfonate respectively, to wash the carbon paste surfaces These surfaces were then used as detectors in a flow system This improved response for species that adsorbed such as tris (1 10 phenantholine) iron (II) and for species which they reported did not adsorb such as NADH and ferricyanide The improvement in response was attributed to a decrease in uncompensated resistance, an increase in reproducibility surface wettability and active area of the surface making the surface more accessible for electroactive species

Previous work by Bard and coworkers (52) on the reductive electrochemistry of methyl viologen (MV) in and premicellar micellar solutions of anionic sodium dodecyl sulfate (SDS) at GC electrodes suggests that not only electrostatic but also other effects such as hydrophobic ones can contribute to surfactant probe interactions At premicellar concentrations they reported coadsorption of negatively charged SDS monomers with the MV cation radical (MV^+) determined from the slope values of CV plots of log of peak currents (i_p) vs log of scan rate (v) Above micellar concentrations of SDS partitioning of MV^+ into the micelles dominated over ion pairing and electron transfer was more difficult because MV^+ was retained in the micelle more strongly than other viologen forms such as the oxidized (MV^{2+}) and the reduced MV This resulted in slower kinetics for the hydrophobic form which partitioned the most into the micelle They concluded that response of MV was more affected by hydrophobic interactions with the micelles in solution that by electrostatic ones and that only micelles in solution were effective in reducing MV$^+$ adsorption

Rusling et al (60) have reported results at basal pyrolytic graphite (PG), coated by covalent attachment of octadecylsilyl (ODS) which was self-assembled with its hydrophobic group extending in a surfactant solution of sodium dodecyl sulfate (SDS) or cetyltrimethyl ammonium bromide (CTAB) Their results suggested formation of a lipid-bilayer assembly resulting from hydrophobic interactions between surfactant tail groups and the ODS film This bilayer film was capable of interacting electrostatically and hydrophobically with analytes (60) They observed improvement in signal to noise (S/N) and better reproducibility at ODS surfactant modified PG over bare BPG This improvement was observed if surfactant probe (electrostatic) interactions were favorable such as for ferrocyanide in CTAB solutions For a hydrophobic probe ferrocene interactions were favorable in SDS and CTAB solutions despite the positive charge of this probe This is consistent with ferrocene remaining primarily in the core of the micelle and therefore, is little effected by electrostatic effects. On the other hand for hydrophilic probes if surfactant and probe had the same charge, a significant inhibition of charge transfer was found They attributed changes in reversiblity of the redox couples to double layer effects including changes in surface concentration of the reactants as a result of adsorption of surfactants at the ODS film with head groups facing solution

Because many biological processes involve electron transfer through a membrane much work has been devoted to the study of similar processes at membrane-solution interfaces Hill et al used surfactants to form membrane-like assemblies at BPG to analyze protein response (61) In general, protein response at unmodified BPG is poor as a result of the lack of activity of this surface In addition, this is a hydrophobic surface poisoned by irreversible protein adsorption In an attempt to improve response for cytochrome c Hill et al modified BPG by self assembling SDS onto to the surface (61) Although this modification enhanced response for cyt c at BPG, problems with decrease in signal with scan number indicated irreversible protein adsorption still existed resulting in surface fouling

Exploitation of the membrane like properties of surfactants has been previously done in our lab for the analysis of small biological molecules such as catecholamines (76) Unlike the previous work by others where permanent modification of the surface was done and surfactants did not remain in solution during analysis in this work surfactants were kept in solution during analysis and were not preadsorbed prior to exposure to analyte solution This strategy relied on direct adsorption of surfactant from the analyte solution as a result of the surface active properties of surfactants In addition, the presence of surfactants in solution resulted in a continuous exchange between surfactant at the surface and in solution which created a dynamic assembly at the surface providing a continuously renewed surface free of memory effects Unlike permanent modifications such as PME SAM and LB methods, dynamic surface modification facilitates probe-surface access without significantly decreasing electron transfer kinetics

This modification strategy was found to improve response for inactive GC, primarily for anionic catecholamines which exhibit poor response at this surface (76). In addition, surfactant modification also improved the reusability of RPG which is otherwise easily fouled by catecholamine adsorption and surface changes during analysis (76). The conclusion of this work is that surfactant-modification of graphite based on dynamic modification is an effective method for controlling and optimizing electrochemical response of biological molecules.

CHAPTER 2
EXPERIMENTAL SECTION

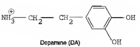
2.1 Reagents

2.1.1 Probes

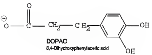
3,4-Dihydroxyphenyl-acetic acid (DOPAC), 3-hydroxytyramine (dopamine, DA) were the two probes investigated at graphite electrodes in surfactant media in the study of surfactant assembly at graphite surfaces. These probes have considerable biological significance. DA is a central nervous system neurotransmitter and DOPAC its metabolite. Due to their small size problems in electrochemical analysis usually encountered with larger biological molecules such as buried redox centers and slow diffusion coefficients could be avoided. These probes were chosen primarily because of their charge where DOPAC (pKa's: 4.22, 9.58, and 12.15 (82)) is negatively charged and DA (pKa 8.92 (83)) is positively charged (Figure 6) at or above pH 7. Both probes exhibit similar oxidation potential with a half-wave potential ($E_{1/2}$) dependent on pH and ca. 0.150 V vs SCE at pH 7 (84). The oxidation of DOPAC and DA involves a two electron/two proton reaction (84). The diffusion coefficients is 6.0×10^{-6} cm^2/s for DA and 5.9×10^{-6} cm^2/s for DOPAC (85).

Graphite surface modification with surfactants for electrochemical detection of proteins was tested with a redox protein, horseheart cytochrome c (cyt c)

Figure 8. The Structure of DOPAC and DA at pH 7



Dopamine (DA)
3-Hydroxytyramine



DOPAC
3,4-Dihydroxyphenylacetic acid

Cyt c is a well studied protein found in the cell mitochondria In the mitochondria it is involved in a one electron transfer reaction In native form cyt c in the oxidized form (Fe^{3+}) The formal potential ($E^{\circ\prime}$) for the reduction of native cyt c depends on the redox partner involved in the reaction For example following the reduction of cyt c (Fe^{2+}) by cyt c reductase a $E^{\circ\prime}$ of 0 030 V vs SCE is observed (86) While a $E^{\circ\prime}$ of 0 010 vs SCE is reported for native solution free cyt c (2) It has a molecular weight of 12 000 daltons with dimensions of 25 x 25 x 35 Å and a known diffusion coefficient in water of 5 x 10^{7} cm^2/s (2) It is a hydrophilic protein with a net positive charge at neutral pH or higher (isoelectric point ≈ 10) due to the presence of lysine groups around the heme edge The prosthetic heme group and the positive lysine residues surrounding this group are illustrated in Figure 9 The rest of the other amino acids are illustrated in blue From this figure the sides corresponding to 35 and 25 Å are also specified All probes were purchased at Sigma Chemical Co

2 12 Surfactants

The aliphatic surfactants used to modify graphite were chosen on the basis of charge and hydrophobicity Figure 10 shows the structure for the surfactants investigated 12 carbon chain length sodium dodecyl sulfate (SDS) (Fisher Scientific Co), 14 carbon chain length tetradecyltrimethyl ammonium bromide (TTAB), 12 carbon chain length dodecyl-trimethyl ammonium bromide (DTAB) and 16 carbon chain length hexadecyltri-methyl ammonium bromide (CTAB) (Sigma Chemical Co) Deoxycholic Acid (DCA) (Sigma Chemical Co) was the

Figure 9. Space Filling Representation of Cytochrome c. The prosthetic heme groups is highlighted in red and the surrounding positive lysine groups are highlighted in green. The remaining amino acids are highlighted in blue. Molecular dimension along the y axis is 35 Å and along the x axis is 25 Å

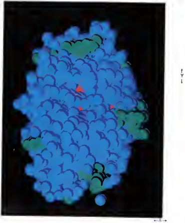

Y
X

Figure 10 Structure of Ionic Aliphatic Surfactants (A) SDS, (B) CTAB

A) $CH_3(CH_2)_{11}OSO_3^- \ Na^+$

SDS (12 carbon)
Sodium Dodecyl Sulfate

B) $CH_3(CH_2)_{15}N(CH_3)_3^+ \ Br^-$

CTAB (16 carbon)
Cetyltrimethylammonium Bromide

Also investigated DTAB (12 carbon) and TTAB (14 carbon) bile surfactant chosen for analysis of the redox protein cyt c. Its structure was shown earlier in Figure 7 (e).

2.13 Solution Conditions

All chemicals were used as received except for cyt c which was purified prior to analysis. Solutions were prepared daily in doubly distilled deionized water. All experiments were run at room temperature at ca. 25 °C. Ionic strength was adjusted with sodium phosphate monobasic and dibasic which were obtained from Mallinckrodt. Phosphate buffer concentrations for catecholamine analysis were kept between 0.05 and 0.50 M at a pH of 7. Solution pH was measured using a Corning pH Meter 130. Charge screening effects of multiply charged cations were investigated with 5 mM magnesium chloride ($MgCl_2$). Catecholamine concentrations ranged between 0.05 and 5 mM. Aliphatic surfactant concentrations were kept above cmc. Anionic surfactant SDS (aggregation number 62, cmc = 2.25 mM (59)) solutions were 10 mM and cationic surfactants CTAB, TTAB, and DTAB (aggregation numbers 78-72, cmc = 1.3 mM (59)) solutions were 3 mM.

Cyt c concentrations ranged between 9 and 200 μM with ionic strength adjusted with phosphate and kept between 10 and 100 mM. DCA concentrations ranged between 0.05 and 5 mM for catecholamine and cyt c analysis. Experiments with DCA surfactants were conducted at pH 8 to ensure ionization of DCA. The original solution pH was raised from 7 to 8 with NaOH (Fisher Scientific Co.)

2.2 Protein Purification Procedure

Cyt c purification was necessary to remove impurities such as 17% of the deamidated forms and the 5 % of the high molecular weight polymeric forms. A Whatman cation exchange column of carboxyl methyl (CM 52) cellulose described by Margoliash et. al. (87) was used. In order to get maximum yield of native cyt c in the desired oxidized form cyt c sample was oxidized with a five time molar excess potassium ferricyanide. The unpurified cyt c and the ferricyanide solution were prepared in a 70 mM phosphate buffer of pH 7.2. The column had an i.d. of 1.5 cm. Resin height was 10 cm. The optimum flow rate was from 0.3 to 0.4 ml/min for a 50 mg sample of cyt c resulting in a total elution volume of 600 ml. With this elution volume the protein polymeric forms remained on the column and were removed when the resin was regenerated in 0.50 M NaOH for several days. Lower flow rates significantly extended the elution time while faster flow rates resulted in large elution volumes without significantly decreasing retention time.

A 70 mM phosphate buffer at pH 7 was used as an eluent providing the fastest elution with baseline resolution. At higher phosphate concentrations separation efficiency was sacrificed. Furthermore problems with high salt concentrations in the final sample prevented using higher ionic strength.

The separation was monitored at 360 nm where cyt c and ferricyanide absorb (87). A chromatogram with 5 peaks of ferrocyanide ca. 50 ml, 3 deamidated forms of cyt c at 150, 175 and 275 ml, and the native cyt c eluting at 300 ml was typically obtained.

The native cyt c was concentrated by Freeze Drying (Labconco Freeze Dryer 8) but the resulting samples had a high ionic strength. In order to decrease the ionic strength, desalting was necessary. This was done by ultracentrifugation using membranes with a molecular weight cutoff of 10 000 Daltons (Centricell 20 Polysciences, Inc) at a rotation speed of 2000 rpm. The sample on the membrane was rinsed several times with deionized water to remove as much salt as possible. The final filtrate was analyzed for phosphate content with 0.10 M ammonium molybdate in 5 M sulfuric acid. The typical phosphate concentrations in the final native cyt c solution were between 10 and 100 mM.

An alternative approach was the ultracentrifugation of the purified sample allowing simultaneous desalting and concentrating of the cyt c sample. Phosphate concentrations by this method were calculated to be approximately 35 mM.

The sample remaining on the membrane was dissolved in doubly distilled deionized water to give final cyt c concentrations between 30 and 200 μM. These were determined by UV analysis at 550 nm using ε = 21 100 (87) for the iron-sulfur chromophore. Rhodamine b (λ_{max} = 550 nm ε = 72,443) was used as a standard in absorbance calibration.

2.3 Electrochemical Apparatus and Procedures

2.31 Electrode Preparation

Rough pyrolytic graphite (RPG) and glassy carbon (GC) electrodes (Electrosynthesis) were constructed by sealing a ca. 0.05 cm^2 piece of RPG or a ca. 3 mm diameter rod of GC in glass tubing with epoxy (Hysol Aerospace and Industrial Products Division). RPG electrodes were resurfaced on 600-grit silicon carbide paper (Mark V Laboratory) using the Buehler Ecomet I Polisher-Grinder and rinsed with deionized distilled water. GC electrodes were polished initially on 600-grit silicon carbide paper and then polished on an alpha a polishing cloth (Mark V Laboratory) with gamma alumina suspensions of particle sizes ca. 0.1 µM (Gamal, Fisher Scientific Co.) using a Buehler Ecomet I Polisher-Grinder. The electrodes were resurfaced prior to each measurement.

Carbon fiber electrodes where glued to a copper wire with silver expoxy (EPO TEK 410 E, Epoxy Technology Inc.). A carbon fiber with the connection to copper wire was placed in a glass capillary and sealed with epoxy (Hysol Aerospace and Industrial Products Division). After the electrode was made the tip of the electrode was cut with a scalpel. The electrode was polished on an alpha a polishing cloth (Mark V Laboratory) with gamma alumina suspensions of particle sizes finer than 0.1 µM (Gamal, Fisher Scientific Co.) using a Buehler Ecomet I Polisher Grinder. The electrode was resurfaced prior to each measurement.

2 32 Electrochemical Methods

The electrode areas as well for the conventional size electrodes were determined using chronocoulometry with a Bioanalytical Systems electrochemical analyzer, BAS 100 and the data were downloaded to an IBM PS/2 Model 50 computer A conventional three electrode set up was employed with a GC or RPG working electrode a 0 8 cm square platinum ribbon auxillary electrode (Sargent), and a saturated calomel (SCE) reference electrode All potentials are reported at room temperature versus SCE Electrode areas were determined in solutions of 4 0 mM potassium ferricyanide $K_3Fe(CN)_6$ (Fisher Scientific Co), in 1 0 mM potassium chloride (Fisher Scientific Co) with diffusion coefficient of D_o = 7 63 x 10^{-6} cm^2/s (88) Solutions were deaerated for at least 15 minutes with nitrogen The potential window was stepped from 0 400 to 0.0 V vs SCE at a pulse width of 250 ms Immediately after placing GC and RPG in solution measurements were taken The electrode areas were determined to be 0 050 ± 4 cm^2 for RPG and 0 060 ± 0 02 cm^2 for GC

The electrode radii of the carbon fiber disk electrodes were determined with the same solution of $K_3Fe(CN)_6$ in 1 0 mM KCl using cyclic voltammetry (CV) experiments with a potential window of 0 400 to 0 0 V vs SCE at a scan rate of 0 100 Vs^{-1} For the ultramicroelectrodes a two electrode set was used with carbon fiber as the working electrode and SCE as the reference The electrode radii calculated were between 2.7 ± 0 7 and 3 1 ± 0 2 μm

At conventional size electrodes CV experiments were conducted at scan rates (v) between 0 010 and 0 100 Vs^{-1} to measure peak currents (i_p) and anodic to cathodic peak separations (ΔE_p) which is the difference between anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) The potential window used for catecholamine analysis was 0 0 to 0 400 V and for protein cyt c analysis was 0 100 to -0 200 V vs SCE

To investigate adsorption at the surface CV experiments were also conducted at v ranging from 10-1000 mVs^{-1} to measure i_p as a function of v Cyt c surface coverage (Γ mol/cm^2) was determined from areas under a CV curve for a 23 μM cyt c in 0 25 mM DCA solution at DCA modified GC In experiments with cyt c background currents were determined from the CV measured currents in the presence of cyt c.

2 4 Fundamentals of Electrochemical Methods

2 41 Electrochemical Response at Conventional Size Electrodes

Measurements of ΔE_p values can be used to determine if the process controlling heterogeneous electron transfer involves adsorption or diffusion In addition the reversibility of those processes can be determined The theoretical ΔE_p value for a diffusion controlled reversible heterogeneous electron transfer is 0 059/n (V), where n is the number of electrons transferred per mole while ΔE_p for an adsorption reversible process is 0 0 V (12) Larger ΔE_p greater than 0 059/n V for a diffusion process and greater than 0 0 V for an adsorption process indicate electron transfer kinetics are slow (e g quasireversible or irreversible)

In the analysis of catecholamines a ΔE_p value of 0.030 V corresponds to a reversible diffusion controlled system since these probes undergo a $2e^-/2H^+$ oxidation (9,15,84). In the analysis of redox protein cyt c, which undergoes a 1e reduction a ΔE_p value of 0.059 V is expected for a diffusion controlled reversible process.

Slope values for plots of log of CV peak currents (i_p) vs log v can be used to investigate probe adsorption. The theoretical i_p value for a diffusion controlled reversible system can be calculated from the equation below

$$i_p = (2.69 \times 10^5) n^{\frac{3}{2}} A D_o^{\frac{1}{2}} v^{\frac{1}{2}} C_o^* \tag{10}$$

and for an irreversible diffusion controlled system from

$$i_p = (2.99 \times 10^5) n (\alpha n_a)^{\frac{1}{2}} A C_o^* D_o^{\frac{1}{2}} v^{\frac{1}{2}} \tag{11}$$

where n is the number of electrons per mole oxidized or reduced. A is the electrode area (cm^2). D_o is the diffusion coefficient (cm^2/s). v is the scan rate defined earlier (V/s). C_o^* is the bulk concentration of the electroactive probe (mol/cm^3). α is the transfer coefficient and n_a is the number of electrons involved in the rate determining step.

For an adsorption controlled process the theoretical i_p values for a reversible and irreversible system are described below. For a reversible system

i_p is:

$$i_p = \frac{n^2F^2}{4RT}\, vA\Gamma_o \tag{12}$$

and for an irreversible system

$$i_p = \frac{n\alpha n_a F^2 A v \Gamma_o}{2\ 718RT} \tag{13}$$

where Γ_o is the surface excess of the electroactive species (mol/cm^2), F is Faraday constant (96 4846 C/equiv), R is the ideal gas constant (8 31441 J/Kmol), and T is the absolute temperature (K) From equations 10-13, plots of log i_p vs log v should be linear and have slope values 0 5 for diffusion ($i_p \propto v^{1/2}$) and 1 0 for adsorption ($i_p \propto v$) independent of electrochemical kinetics

From the above equations for diffusion limited processes (eqs 10 and 11), i_p values could be used to determine changes probe concentration and i_p values for adsorption process (eqs 12 and 13) can be used to determine surface coverage ΔE_p values can be used to determine changes of electron transfer kinetics which reflect changes in probe-surface distance where an increase in ΔE_p is consistent with an increase in probe-surface distance

2.42 Electrochemical Response at Ultramicroelectrodes

When the electrode size is decreased to the micrometer scale, many changes occur in the voltammetric behavior These changes can lead to dramatic improvements in the quality of electrochemical data and can facilitate several types of experiments that were previously impossible The major areas of improvements include increased temporal resolution, decreased sensitivity to the effects of solution resistance and the ability to make spatially resolved chemical measurements (89 90)

The diffusion layer is the region adjacent to the electrode in which the chemical composition is altered by the electrolysis process Mass transport through this region is one of the factors determining the shape of the voltammogram and the magnitude of faradaic current The exact form of the current depends on the geometry of the electrode However certain generalizations can be made At values of Dt/r_o^2 greater than one where D is the diffusion coefficient (cm^2/s) of the electroactive species t is the electrolysis time (s) and r_o is the electrode radius (cm) the dimensions of the double layer are greater than of the electrode and radial diffusion mass transport is observed This leads to a sigmoidally shaped CV where at conventional size electrode a peak shaped CV is observed due to linear diffusion (90)

At higher v (> 0 500 Vs^{-1}) when the value of Dt/r_o^2 is less than one the dimensions of the double layer are less than of the electrode and response has a peak shape due to linear diffusion mass transport similar to that observed at conventional size electrodes However, the experiments conducted here in the analysis of catecholamines were conducted at v less than 0 100 Vs^{-1} to ensure sigmoidal response (radial diffusion mass transport) (90) Under these conditions the theoretical limiting current (i_l) for an ultramicro disk electrode can be calculated from the following equation

$$i_l = 4nFDCr_o \tag{14}$$

where all terms have been previously defined (89,90) The i_l (eq 14) at a disk electrode under radial diffusion conditions is independent of v and, therefore, independent of electron transfer kinetics However the plateau potential (E_p) the potential where i_l is reached and response plateaus can be used as a measure of difficulty or ease of electron transfer For example for an oxidation process an increase in E_p to more positive potentials is consistent with an increased difficulty of electron transfer In addition, in the case of very slow kinetics steady state response is not reached and not well defined plateau of current is observed

CHAPTER 3
SURFACE MODEL FOR IONIC ALIPHATIC SURFACTANT ASSEMBLY AT GRAPHITE

The focus of this chapter is the investigation of aliphatic surfactant assembly at graphite in analysis of catecholamines and the development of a surface model for ionic aliphatic surfactant assembly at two graphite surfaces RPG and GC The model was developed using surfactants of different charge and carbon chain length such as cationic ammonium bromide surfactants of 12, 14 and 16 carbon chain lengths and 12 carbon chain length anionic sodium dodecyl sulfate (SDS) to distinguish between the importance of electrostatic and hydrophobic effects on surfactant assembly at graphite in the response of surface active catecholamines The model was tested with ionic catecholamines, 3,4-dihydroxyphenyl-acetic acid (DOPAC) and 3-hydroxytyramine (DA) The feasibility of forming surfactant aggregates at these surfaces directly from solution without the need for permanent surface modification will be demonstrated This dynamic assembly will prove to be effective in reducing surface changes while not hindering electron transfer Furthermore these results will indicate that electrostatic effects are more important for surfactant assembly at GC than at RPG where hydrophobic effects are more important.

Structural differences between RPG and GC result in the different effects of surfactants on probe response. In order to optimize response at graphite the surfactant, the nature of the probe and the surface structure must all be considered. It will be shown that aggregates of ionic surfactant at the graphite electrodes allow the control and optimization of response of these surfaces by providing a favorable environment for biological molecules such as catecholamines. Furthermore in surfactant solutions improvements in the sensitivity of inactive GC and in the reproducibility of active RPG will demonstrated.

To characterize the response of the probes in the absence of surfactant modification the electrochemical response of DA and DOPAC at GC and RPG was investigated first. The electrochemical behavior of these probes as well as surfactant effect on probe response at GC and RPG are discussed in detail below.

3.1 Results and Discussion for Bare Surfaces

3.1.1 Catecholamine-GC Interactions

Electrochemical kinetics of anionic DOPAC and cationic DA are both slow at GC (14,76). The slow kinetics are apparent in cyclic voltammetry (CV) experiments from the large differances between anodic and cathodic peak potentials (ΔE_p) which are shown in Table 2. ΔE_p values of ca. 30 mV are expected for a fast two-electron (reversible) couple (12). Under the experimental conditions of Table 2 peak currents (i_p) for DOPAC and DA are expected to be equal and can be easily predicted from the equation for CV currents for

Table 2 CATECHOLAMINE RESPONSE AT BARE AND SURFACTANT-MODIFIED GC

SOLUTION[a]	ΔE_p[b] (mV)	i_p[c] (μA)	$i_{p\,theo}$[d] (μA)	m[e]
DOPAC/BUFFER	250 ± 3	0.24 ± 0.03	0.33	0.45 ± 0.05
DOPAC/DTAB(12)	204 ± 7	0.41 ± 0.01		0.46 ± 0.04
DOPAC/TTAB(14)	131 ± 12	0.43 ± 0.03		0.48 ± 0.02
DOPAC/CTAB(16)	110 ± 9	0.43 ± 0.05		0.43 ± 0.03
DA/BUFFER	169 ± 33	0.39 ± 0.02	0.33	0.42 ± 0.02
DA/SDS(12)	156 ± 11	0.40 ± 0.03		0.38 ± 0.01
DOPAC/SDS(12)	512 ± 42	0.17 ± 0.05		

[a] Buffer solutions prepared with 0.5 mM probe and 0.5 M pH 7 phosphate buffer surfactant solutions were prepared as buffer solutions with 3 mM cationic surfactants and 10 mM anionic surfactants

[b] CV experiments conducted at a ν of 0.100 Vs^{-1}

[c] Oxidation peak currents normalized over electrode area of 0.06 cm^2 and 0.5 mM probe concentration.

[d] Theoretical peak currents for a two electron diffusion controlled irreversible system calculated using equation 11 normalized over electrode area and probe concentration as in [c]

[e] Slopes for CV plots of log i_p vs log ν with correlation coefficient of 0.98-1.00 ν ranged between 0.010 and 1.0 Vs^{-1}

irreversible two electron system (12) The experimental and theoretical i_p values for DOPAC and DA calculated using equation 11 are listed in Table 2 The results show that i_p for DA is larger than predicted indicating that some preconcentration at the surface may in fact be occurring The i_p values for DOPAC are significantly lower than predicted, suggesting lower concentration at the surface Cationic preconcentration effects have been previously reported in other studies of catechols at inactive GC (14-16)

Electrostatic interactions may be responsible for these results, although surface interactions of DA are not detected from slopes of log i_p vs log scan rate (v) plots listed in Table 2 For adsorbing species theoretical slope values of ca. 1.0 are expected (12) The slope values of the plots of log i_p vs log v listed in Table 2 are below 0.5, the theoretical value for diffusion controlled process (12) Slope values below 0.5 may indicate some hindrance or partial blocking of electrodes, where the electroactive area of the electrode is not the same as the calculated geometric area (72) It has been reported that when hydrophobic interactions at GC are strong, adsorption is detected (52) Furthermore, hydrophobic interactions, have been reported to dominate over electrostatic when both are present (52) The results indicate that hydrophobic interactions of DA and DOPAC must not be strong at GC since no adsorption was evident from slopes of plots of log i_p vs log v The importance of electrostatic interactions for catecholemine response at GC is evident from these results

3 12 Catecholamine-RPG Interactions

Adsorption of DOPAC and DA at RPG has been reported to occur irreversibly causing problems with reproducibility (76) ΔE_p values listed in Table 3 at RPG in buffer are less than 30 mV which is the theoretical value for a diffusion controlled two electron process (eq 10) (12) The slope values in Table 3 of log i_p vs log v plots are greater than 0 5 (expected for a diffusion controlled process) (12) These results provide supporting evidence for DOPAC and DA adsorption at RPG The interactions involved in adsorption of DOPAC and DA at RPG are not totally clear However if electrostatic effects were controlling adsorption of DA and DOPAC negatively charged DOPAC should not adsorb at RPG due to a high density of surface oxides which gives a greater negative charge at this surface than at GC The results in Table 3 however indicate adsorption of DOPAC on RPG

In view of similar ΔE_p values for DOPAC and DA listed in Table 3, which indicate that their kinetics at RPG are similar the currents for both probes should be similar However i_p of DA at RPG is significantly larger than i_p of DOPAC This indicates higher concentration of DA at the surface Since positively charged DA can be expected to electrostatically interact with the oxide rich surface negatively charged DOPAC-surface interactions must in fact be less favored From the results it is clear however that in addition to electrostatic effects other surface features such as roughness and lattice orientation must contribute to the observed high surface concentration of DOPAC Since RPG is

Table 3 CATECHOLAMINE RESPONSE AT BARE AND SURFACTANT-MODIFIED RPG

SOLUTION[a]	ΔE_p[b] (mV)	i_p[c] (Acm^{-2}M^{-1})	m[d]
DOPAC/BUFFER	20 ± 9	1.01 ± 0.11	0.65 ± 0.02
DOPAC/DTAB(12)	32 ± 1	1.04 ± 0.08	0.50 ± 0.05
DOPAC/TTAB(14)	33 ± 1	1.07 ± 0.04	0.53 ± 0.03
DOPAC/CTAB(16)	43 ± 4	0.83 ± 0.04	0.57 ± 0.02
DA/BUFFER	27 ± 2	1.46 ± 0.12	0.62 ± 0.01
DA/SDS(12)	43 ± 4	0.80 ± 0.05	0.56 ± 0.02

[a] Buffer solutions prepared with 0.5 mM probe and 0.5 M pH 7 phosphate buffer; surfactant solutions were prepared as buffer solutions with 3 mM cationic surfactants and 10 mM anionic surfactants.

[b] CV experiments ran at a ν of 0.100 Vs^{-1}

[c] Oxidation peak currents normalized over electrode area of 0.05 cm^2 and 0.5 mM probe concentration

[d] Slopes for CV plots of log i_p vs log ν with correlation coefficient of 0.98-1.00. ν ranged between 0.010 and 1.0 Vs^{-1}

more hydrophilic than GC hydrophobic effects by themselves can not account for the observed adsorption

3 2 Results and Discussion for Surfactant Modified Surfaces

3 21 Surfactant Effect on Catecholamine Response at GC

Anionic SDS and cationic DTAB the surfactants used in this work both have 12-carbon chain lengths and singly charged head groups The electroactive probes used in testing surfactant interactions at GC were again DOPAC and DA are negatively and positively charged respectively, at pH 7 Both probes also have some degree of hydrophobicity Table 2 lists i_p and ΔE_p values from CV experiments As shown in Table 2, i_p values for DOPAC in the presence of DTAB are much greater than predicted for an electrochemically irreversible probe (eq 11) and therefore much greater than the i_p values at bare GC In addition the ΔE_p value for DOPAC decreases following DTAB addition The results point to the presence of DTAB in solution and at the surface favoring DOPAC response at GC Table 2 also lists i_p and ΔE_p values for DA in the presence of SDS No significant affect on DA response was observed following SDS addition

In order to verify that SDS assembles at GC it was necessary to investigate the response of anionic DOPAC since DA response was not significantly effected in SDS solutions In SDS solutions i_p values measured for DOPAC are in fact below the theoretical and experimental values at bare GC (Table 2) In addition ΔE_p values increase significantly indicating an increase in probe-surface distance The inability of DOPAC in SDS surfactant solutions to access GC and the increase of anionic charge near the surface is evident from the increased electrode hindrance observed for DOPAC

Previous work with surfactants in a Langmuir-Blodgett trough provides evidence of decreased rates of electron transfer following increasing aggregate thickness which is attributed to greater electrode hindrance (72) The effect of increasing carbon chain length on the electrochemical response of GC, however supports a somewhat different model for the effect of surfactants on graphite electrode response Increasing carbon chain length of the cationic surfactant from 12 (DTAB), to 14 (TTAB) and finally 16 carbons (CTAB) results in further improvement in kinetics of DOPAC as is clear from the decrease in ΔE_p values seen in Table 2 which occurs without further change in i_p Increasing carbon chain length of the surfactant should increase the stability of the surfactant layer As a result hydrophobic interactions between packed surfactant hydrocarbon chains can result in more efficient self interactions and interactions with DOPAC allowing it better (closer) access to GC (72) Clearly hydrophobic as well as electrostatic interactions must be contributing to the response Unlike what has been previously reported for other surfaces where the surfactant hindered the response of electroactive probes the presence of cationic surfactant at GC has rendered the surface more active for otherwise non adsorbing anionic DOPAC

Furthermore even though anionic SDS does not alter the sensitivity of the response for DA at GC it does increase the reproducibility of ΔE_p measurements and significantly decreases DOPAC response Together the results indicate that both anionic and cationic surfactants affect the response of GC However, only cationic surfactants improve the response and the response enhancement is most significant with the longer carbon chain length surfactant, CTAB

3 22 Surfactant Effect on Catecholamine Response at RPG

When electroactive probes adsorb at the electrode surfaces, slower rates of electron transfer and lower currents are observed following surfactant addition The decrease in response has been attributed to electrode hindrance caused by the adsorbed surfactant layer DOPAC and DA both adsorb at RPG and therefore the addition of surfactant to solutions containing these probes was expected to result in lower response because the surfactant was expected to limit probe adsorption

Table 3 shows i_p and ΔE_p values for DOPAC and DA in surfactant solutions at RPG The addition of surfactants to solutions of these probes results in a small increase in ΔE_p No change in peak currents for DOPAC is observed in the presence of 12 and 14-carbon chain length cationic surfactants which indicates that concentration of DOPAC at the surface does not significantly differ from that at the bare RPG The i_p decreases when the 16 carbon chain length CTAB is added In CTAB solutions ΔE_p values increase above those measured in shorter carbon chain length cationic surfactant solutions but ΔE_p values still indicate reasonably fast kinetics

The ΔE_p values in cationic surfactant solutions listed in Table 3 for DOPAC are representative of values for quasi reversible two electron system in the absence of adsorption. At RPG following surfactant addition at RPG, slopes of ca. 0.5 of log i_p vs log v plots provide evidence for a decrease in DOPAC adsorption. This together with ΔE_p values indicates that the surfactant does not significantly change the probe kinetics and that the increase in ΔE_p is largely due to the decrease in adsorption.

For DA in SDS solutions, ΔE_p along with slope values of log i_p vs log v plots in Table 3 are representative of values for a quasi-reversible system with no adsorption and are comparable in magnitude to i_p and ΔE_p values for DOPAC in CTAB solutions. The observed changes toward diffusional processes for DA at RPG in SDS solutions suggest a decrease in probe-surface interactions without a significant change in the rate of electron transfer.

The addition of surfactant to solutions was expected to alter currents and peak potentials at RPG only if the surfactant sufficiently altered the surface to prevent probe interactions with the surface. It is apparent from the results that anionic and long chain cationic surfactants do limit catecholamine interactions with RPG, consequently reducing probe response. The cationic surfactants especially of the short chain lengths are, however, less effective in changing the response. As a result, in these cationic surfactant solutions, ΔE_p values for DOPAC are representative of reasonably fast kinetics and the i_p values are similar to those measured at the bare surface. When cationic surfactant carbon chain length is increased to 16 carbons (CTAB), a more efficient surface blockage makes the area effectively smaller and increases probe distance to the surface as is evident from the decrease in i_p and an increase in ΔE_p for DOPAC This decrease in DOPAC response in 16 carbon CTAB is similar to that observed for DA in 12 carbon SDS Similar ΔE_p and i_p values in Table 3 for DOPAC/CTAB and DA/SDS solutions suggest that the DA distance from RPG in SDS solutions is similar to that of DOPAC in CTAB solutions Furthermore the surface concentrations of each probe in surfactant solutions (as measured by i_p values) in Tables 2 and 3 are similar This can occur if SDS does not access the surface efficiently resulting in a probe-surface distance similar to that observed for the longer chain cationic surfactants (CTAB)

3 23 Analytical Significance of the Results

Surfactants have a different effect on probe response at GC and RPG due to the different surface structure and probe-surface interactions The addition of surfactants primarily longer carbon chain length cationic surfactants, improves reproducibility and allows RPG to be reused without resurfacing This of course, occurs with some sacrifice in sensitivity due to the elimination of probe adsorption The relevant results are summarized in Table 4

Repetitive cycling at RPG in DOPAC and DA solutions in the absence of surfactants is followed by a decrease in i_p as shown in the results in Table 4, indicating surface changes are occurring The results for the same measurements conducted in solutions containing CTAB (DOPAC) and SDS (DA), also listed in Table 4 and indicate that the addition of surfactant makes the electrode reusable without the need for resurfacing although i_p decreases for

Table 4 RESPONSE AT BARE AND SURFACTANT-MODIFIED RPG AS A FUNCTION OF SCAN NUMBER

SOLUTION	i_{pa}[a] ($Acm^{-2}M^{-1}$)	SCAN NUMBER
DOPAC/BUFFER[b]	1.48 ± 0.11	1
	1.21 ± 0.13	2
	1.00 ± 0.04	3
DOPAC/CTAB[c]	0.76 ± 0.04	1
	0.77 ± 0.04	2
	0.76 ± 0.05	3
DA/BUFFER[b]	1.76 ± 0.13	1
	1.41 ± 0.18	2
	1.18 ± 0.10	3
DA/SDS[c]	0.65 ± 0.04	1
	0.66 ± 0.05	2
	0.65 ± 0.03	3

[a] Oxidation peak currents normalized over electrode area (0.05 cm^2) and probe concentration (0.5 mM) at a $v = 0.100\ Vs^{-1}$

[b] 0.5 mM probe in 0.5 M pH 7 phosphate buffer

[c] As in [b] with the addition of 3 mM CTAB and 10 mM SDS respectively

both DOPAC and DA compared to the values measured at the bare surface.

At GC the improvement in precision is not observed due to the already good reproducibility of current measurements at the bare surface. This is the case since the probes investigated do not adsorb at this surface allowing this electrode to be reused without resurfacing. However, due to the importance of electrostatic interactions at this surface, anionic probes do not access this surface efficiently and, therefore, exhibit poor response. Figure 11 qualitatively demonstrates the improvement in CV sensitivity of GC in the presence of CTAB where the currents of DOPAC increase and the ΔE_p decreases in 3 mM CTAB solutions. In addition, the results in Table 5 demonstrate the increase in GC sensitivity to DOPAC response in the presence of CTAB. Here, i_p values for 0.050 mM DOPAC are observed in the presence of CTAB where at the bare surface no response is observed for such DOPAC concentrations.

3.3 Surfactant Self-Assembly at Graphite

3.31 Surfactant-GC Interactions

At GC, electrostatic interactions with the surface appear to be most important in controlling surfactant assembly, and, therefore, surfactants are expected to adsorb head on at the surface forming a bilayer-like structure as shown in Figure 12. The results in Tables 2 and 3 suggest that such a bilayer-like assembly is favored for cationic surfactants which can interact more favorably with this surface and is not favored for anionic surfactants. The quality of cationic surfactant assembly at GC appears to improve with increasing carbon chain

Figure 11 CV of DOPAC at the Bare and Surfactant-Modified GC
v = 0.100 Vs^{-1}, 0.50 mM DOPAC and 3 mM CTAB in 0.50 M phosphate buffer at pH 7 with an electrode area of 0.06 cm^2

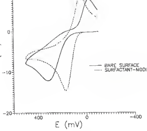

10
0
-10
-20
I (uA)
400
0
-400
E (mV)
— BARE SURFACE
---- SURFACTANT-MODIFIED

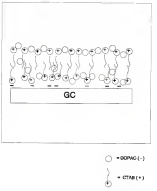
Table 5 IMPROVEMENTS IN SENSITIVITY AT SURFACTANT MODIFIED GC

[DOPAC] mM	i_p(BUFFER)[a] (µA)	i_p(CTAB)[b] (µA)
0.05	NO SIGNAL	0.96 ± 0.05
0.20	NO SIGNAL	4.5 ± 0.1
0.35	1.00 ± 0.03	6.8 ± 0.6
0.50	2.8 ± 0.2	10.4 ± 0.3

[a] Oxidation peak currents for 0.50 M DOPAC in pH 7 phosphate buffer measured at CV v = 0.100 Vs^{-1}

[b] Same as [a] with the addition of 3 mM CTAB

Figure 12. Model for Cationic Surfactant (CTAB) Assembly at GC
The model is for visual purposes only


GC
= DOPAC (-)
= CTAB (+)

length resulting in a more compact assembly at the surface which favors partitioning of probes with hydrophobic moieties thus decreasing surface distances This model is confirmed by the improvement in DOPAC response following an increase in surfactant carbon chain length

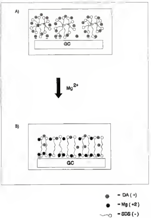
DOPAC in anionic surfactant solutions can not access the GC surface closely as is evident from the dramatic decrease in DOPAC response in SDS solution indicating probe distance to GC is significantly increased in the presence of SDS The assembly of SDS at GC, shown in the next chapter (Figure 14 (A)) is expected to be more disordered and micelle-like

3 32 Surfactant-RPG Interactions

Despite the greater negative charge density of RPG than GC other interactions such as hydrophobic ones are important since both probes adsorb at this surface. The greater surface concentration of DA than DOPAC, however, indicates some preferential cation interactions also occur at RPG as at GC but are less important to good response than at GC

Assembly of cationic surfactant at this surface is better than anionic surfactant assembly, similar to the effects observed at GC Due to greater surface heterogeneity as well as greater surface roughness of RPG than GC, surfactant assembly at this surface can be expected to be more disordered with a proposed micelle-like structure as shown in Figure 13 This is evident from the results, where longer carbon chain length cationic surfactants were necessary for eliminating probe adsorption at RPG while at GC short chain length cationic

Figure 13 Model for Ionic Surfactant (CTAB TTAB DTAB,SDS) Assembly at RPG. This model is for visual purposes only


A)
GC
Mg^{2+}
B)
GC
● = DA (+)
● = Mg (+2)
~o = SDS (-)

surfactants were effective in improving response At RPG shorter carbon chain length surfactants can be expected to form micelle-like assemblies as a result of weaker lateral hydrophobic interactions between neighboring surfactant tail groups and the heterogeneous surface Changing cationic surfactant hydrophobicity by increasing carbon chain length increases lateral hydrophobic interactions between surfactant tail groups and thus increases the order of the aggregates at the surface

The assembly of SDS at RPG is expected to be micelle-like similar to that observed at GC The assembly of anionic SDS is expected to be further away from the surface than cationic surfactants as evident from SDS effectiveness in preventing probe adsorption at RPG In addition, similar probe-RPG distance in 12 carbon SDS and 16 carbon CTAB solutions confirms the inability of SDS to access the surface closely

3 4 Conclusions

The feasibility of forming surfactant aggregates at graphite surfaces directly from solution without the need for tedious methods such as Langmuir Blodgett methods has been shown In addition unlike at other modified surfaces, this dynamically modified surface does not limit electron transfer allowing continuous exchange of the probe between the surface and solution Ewing and Wallingford (75) showed a decrease in electrochemical response for catecholamines such as DA in anionic micellar media which they attributed to changes in probe diffusion coefficients as a result of partitioning of probes into the micelles We did not observe such effects. In fact, diffusion coefficients of catecholamines have been shown not to change significantly in micellar media (76).

The results are consistent with head on surfactant-surface interactions at two types of graphite surfaces, inactive GC and active RPG. Differences in assembly structure are expected due to structural differences between the surfaces, where at smooth GC a more ordered assembly is expected than at rough RPG. The effect of surfactants on probe response varies at these surfaces because of different probe surface and surfactant-surface interactions. Therefore, in addition to choosing the surfactant, the structure of the surface and the nature of the probe must be considered in order to optimize response.

These results indicate that improvement of probe response occurs in surfactant media as a result of controlling the surface microenvironment. Improvements in sensitivity of inactive GC and the stability of response at RPG, with minimal sacrifice in sensitivity were observed in ionic surfactant media. Inactive GC can be made more active without exposing edge planes and the activity of RPG is not compromised. The information gained in the study of surfactant-modified graphite surfaces can be used to elucidate several key properties of graphite surfaces which must be considered for controlling and optimizing probe response at these surface. For example, from these results it is clear that for good response electrostatic effects are important at GC and hydrophobic ones are important at RPG.

CHAPTER 4
OPTIMIZATION OF RESPONSE FOR SMALL BIOLOGICAL MOLECULES IN SOLUTIONS OF IONIC ALIPHATIC SURFACTANTS

Previous results with aliphatic surfactants indicated that despite differences in surface roughness and surface heterogeneity surfactants assemble at GC and RPG head on as a result of the significant hydrophilic nature of these surfaces which have a net negative surface charge at pH 7 Assembly of cationic surfactant at both surfaces was shown to be better than for anionic ones However assembly of negatively charged surfactants appears to be better at RPG than at GC despite the greater density of negative surface groups at this surface These results indicated that hydrophobic effects are important to assembly and response at RPG while electrostatic ones are important at GC

Surfactant modification of graphite was shown to be favorable for sensitive and stable response of small biological probes e g , catecholamines The focus of the work described in this chapter is further improvement of response for catecholamines at the surfactant-modified surfaces To achieve this objective, both probe surfactant and surfactant-surface interactions must be considered and optimized If electrostatic interactions drive assembly interactions for anionic surfactant could be improved by effective charge screening while exposure of these negative groups is favorable for cationic surfactant assembly This could be done by controlling ionic strength or by addition of multiply charged cations which are more efficient in charge screening the surface groups

Prior to optimizing surfactant-graphite interactions catecholamine response at graphite was investigated as a function of ionic strength and cation charge These results will show that control of ionic strength and charge screening does not improve response for catecholamines as significantly as surfactant modification presumably because surfactants can provide a favorable hydrophobic and hydrophilic (electrostatic) environment which is apparently necessary for good catecholamine response at graphite A detailed discussion of ionic strength effects on catecholamine response and on surfactant-surface interactions will follow

4 1 Ionic Strength Effects on Catecholamine-Graphite Interactions

4 11 Double Layer Effects on Catecholamine-GC Interactions

The response of anionic 3 4-dihydroxyphenyl acetic acid (DOPAC) at GC was shown earlier not to be very sensitive due to repulsion of the probe by the anionic surface groups (14-16) Table 6 lists the values of the difference between the anodic and cathodic peak potentials (ΔE_p), and peak currents (I_p) at different ionic strengths Increases in ionic strength result in an increase in I_p and a decrease in ΔE_p, e g higher surface concentration of DOPAC and closer surface access Addition of electrolytes not only increases the ionic strength but also changes the composition of ions in the double layer Electrolytes with multivalent cations can be expected to be more effective in screening anionic

Table 6 EFFECT OF DOUBLE LAYER ON CATECHOLAMINE RESPONSE AT BARE GC

SOLUTION[a]	[ELECTROLYTE][b] mM	i_p[c] A/cm²M	ΔE_p[c] (mV)
DOPAC	50	0.27 ± 0.02	421 ± 10
DOPAC	500	0.38 ± 0.02	270 ± 17
DOPAC/$MgCl_2$[d]	65	0.44 ± 0.02	193 ± 5
DA	50	0.51 ± 0.03	124 ± 13
DA	500	0.41 ± 0.01	193 ± 20
DA/$MgCl_2$[d]	65	0.46 ± 0.02	113 ± 4

[a] Probe concentrations were 0.50 mM

[b] Phosphate concentration at pH 7

[c] Oxidation peak currents normalized over electrode area (0.05 cm²) and CV experiments were conducted at $v = 0.100\ Vs^{-1}$

[d] $MgCl_2$ = 5 mM concentration in addition to 50 mM phosphate at pH 7

surface charge (13) The results of DOPAC response at GC in which ionic strength was adjusted with $MgCl_2$ are summarized in Table 6 When $MgCl_2$ is added a significant increase in i_p and a decrease in ΔE_p is observed for DOPAC consistent with the expected effect of divalent cations on charge screening and double layer compactness

An increase in ionic strength should decrease dopamine (DA)-GC interactions decreasing i_p (lowering surface concentration) and increasing ΔE_p (increasing distance to the surface) because of the competition of electrolyte cations for the negative surface groups Results in Table 6 verify this supporting the importance of electrostatic interactions to DA response at GC Addition of multivalent cations was also expected to decrease amount of DA at GC since multivalent cations could compete for the surface more efficiently than monovalent ones The results in Table 6 indicate that i_p (concentration) for DA decreases following addition of $MgCl_2$ while no significant effect on ΔE_p (distance) is observed These results are consistent with the importance of favorable electrostatic interactions to good response for catecholamines at inactive GC

4 12 Double Layer Effects on Catecholamine-RPG Interactions

Earlier results showed that DOPAC-RPG and DA-RPG interactions are more complex than probe-GC interactions not simply electrostatic, and rely on the close probe-surface access At high ionic strength both probes should be closer to RPG due to a more compact double layer The results in Table 7 confirm this, since a decrease in ΔE_p and an increase in i_p values following an increase in ionic strength is observed for both DOPAC and DA

Addition of multivalent cations can be expected to decrease electrostatic interactions for DA and increase those for DOPAC at RPG However since previous results indicated that other interactions such as hydrophobic ones are important, the effect on probe response should be small The results in Table 7 indicate that i_p, ie surface concentration, for DA at RPG decreases following addition of $MgCl_2$ while i_p for DOPAC slightly increases For DOPAC a slight increase in ΔE_p is observed following $MgCl_2$ addition indicating a slight increase in probe-RPG distance However, for DA no significant change in ΔE_p was observed

Another interesting feature of the results in Table 7 is the similarity in ΔE_p values for DA and DOPAC at RPG in solutions of comparable ionic strength The similar and low ΔE_p values at high ionic strength demonstrate the ability of both probes to access this surface closely However, surface concentrations as measured by i_p differ for the two probes with values for DA remaining higher which is consistent with some cation preconcentration at the surface

The overall effect of ionic strength and cation charge was less apparent at this surface than at GC, confirming that electrostatic effects are not as important at this surface as at GC Other interactions with the surface such as hydrophobic ones are more important for good response at RPG

Table 7 EFFECT OF DOUBLE LAYER ON CATECHOLAMINE RESPONSE AT BARE RPG

SOLUTION[a]	[ELECTROLYTE][b] (mM)	i_p[c] (A/cm²M)	ΔE_p[c] (mV)
DOPAC	50	0.61 ± 0.03	47 ± 2
DOPAC	500	1.23 ± 0.10	20 ± 9
DOPAC/$MgCl_2$	55[d]	0.68 ± 0.11	56 ± 4
DA	50	0.98 ± 0.06	47 ± 2
DA	500	1.36 ± 0.05	25 ± 5
DA/$MgCl_2$	55[d]	0.69 ± 0.04	51 ± 3

[a] Probe concentrations were 0.50 mM

[b] Phosphate concentration at pH 7

[c] Oxidation peak currents normalized over electrode area (0.05 cm²) and CV experiments were conducted at $\nu = 0.100\ Vs^{-1}$

[d] $MgCl_2$ = 5 mM concentration in addition to 50 mM phosphate at pH 7

4.2 Ionic Strength Effects on Surfactant-Graphite Interactions

4 21 Double Layer Effects on Surfactant-GC Interactions in Catecholamine Analysis

The results in Table 8 show that the response of DOPAC at GC in cetyltrimethylammonium bromide (CTAB) solutions is best at low ionic strength which favors surfactant-surface and probe-surfactant interactions This is evident from the higher i_p and the lower ΔE_p values Furthermore, higher i_p and lower ΔE_p values in the presence of surfactant confirm that surfactant assembles at the surface and that such an assembly and the hydrophobic environment it provides is important for good DOPAC response at GC The response of DOPAC (in CTAB solution at GC) decreases following addition of $MgCl_2$ as evident from the decrease in i_p and an increase in ΔE_p (Table 3) which indicates a decrease in surfactant assembly at this surface

The results in Table 8 for DA in sodium dodecyl sulfate (SDS) solutions indicate that when ionic strength is increased i_p of DA increases at GC approaching values in the absence of SDS, but with lower ΔE_p (Table 1) Lower ΔE_p values in the presence of SDS confirm more effective probe access to the surface However, similarly to what was observed in the absence of SDS ΔE_p for DA increases with increasing ionic strength (Table 8) At high ionic strength, charge screening of SDS head groups or larger aggregate size may cause an increase in DA-surface distance Addition of $MgCl_2$ to DA in SDS solutions resulted in a decrease in ΔE_p at GC with little effect on i_p (Table 8) These results indicate closer access of GC for DA presumably due to better assembly of SDS

Table 6. DOUBLE LAYER EFFECT ON SURFACTANT-GC INTERACTIONS

SOLUTION[a]	[ELECTROLYTE][b] (mM)	i_p[c] A/cm^{2}M	ΔE_p[c] (mV)
CTAB/DOPAC	50	0.51 ± 0.02	80 ± 2
CTAB/DOPAC	500	0.43 ± 0.05	110 ± 9
CTAB/MgCl$_2$/DOPAC	55[d]	0.46 ± 0.01	103 ± 2
SDS/DA	50	0.32 ± 0.01	122 ± 3
SDS/DA	500	0.40 ± 0.03	156 ± 11
SDS/MgCl$_2$/DA	55[d]	0.38 ± 0.03	115 ± 5

[a] Probe concentrations were 0.50 mM with 3mM CTAB solutions containing DOPAC and 10 mM SDS solutions containing DA.

[b] Phosphate concentration at pH 7

[c] Oxidation peak currents normalized over electrode area (0.06 cm^2) and CV experiments were conducted at $v = 0.100$ Vs^{-1}.

[d] MgCl$_2$ concentration = 5 mM in addition to 50 mM phosphate at pH 7

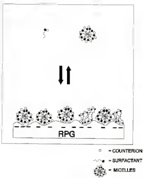
at GC as a result of effective charge screening by Mg^{2+} Again these results are consistent with the importance of electrostatic effects at this surface

Increasing ionic strength and the resultant charge screening causes a less effective assembly of cationic surfactants at GC However anionic surfactants appear to assemble more efficiently Two processes may be responsible more effective formation of larger micelles through effective screening of surfactant head groups minimizing head group repulsion and/or increased surface charge screening, resulting in enhancement in interactions for anionic species and a decrease in interactions for cationic ones Clearly the latter effect is important in cationic surfactant solutions $MgCl_2$ has a similar effect as ionic strength on SDS and CTAB assembly again a consequence of charge screening effects As shown in Figure 14 (B) Mg^{2+} may screen the negative charge (surface and surfactant) improving assembly of anionic SDS Mg^{2+} reduces assembly of CTAB at GC

4 22 Double Layer Effects on Surfactant RPG Interactions in Catecholamine Analysis

At bare RPG where electrostatic interactions had less effect on probe response than at bare GC, assembly of surfactants was not expected to be strongly affected by electrolytes present in solution Furthermore in surfactant assembly at RPG hydrophobic effects (rather than electrostatic) should play an important role surfactant assembly at the surface may be favored over that of electrolyte ions The results in Table 9 confirm this since only a slight decrease in DOPAC response was observed in CTAB solutions with increasing ionic

Figure 14 Model for Anionic Surfactant Assembly at GC (A) SDS assembly, (B) $MgCl_2$ effect on SDS assembly at GC This is a cartoon representation for visual purposes only


RPG
= COUNTERION
= SURFACTANT
= MICELLES

strength or following addition of $MgCl_2$

At RPG CTAB and SDS were shown earlier to reduce probe adsorption in a similar manner Results in Table 9 indicate that DA response at RPG in SDS solutions is better at lower ionic strength Here as with CTAB the effect of ionic strength on response is very small $MgCl_2$ addition to DA in SDS solutions also had very little effect on DA response at RPG as shown in the results in Table 9 The results in Table 9 indicate a slight decrease in DA response following addition of $MgCl_2$ Furthermore the effect of $MgCl_2$ addition on DA response was similar to that observed for DOPAC in CTAB solutions following $MgCl_2$ addition indicating the importance of other interactions besides electrostatic ones to good response at RPG In addition the overall effect of electrolytes on the response of RPG and GC in surfactant solutions is much less significant than at the bare surfaces These results indicate that surfactant assembly is favored over other species in solution making them fairly insensitive to double layer composition

4 3 Conclusions

Results obtained here show that at RPG both electrostatic interections (due presumably to negative oxide groups) and hydrophobic interactions (a possible result of a higher basal plane density) are important In the reactions of catechols hydrophobic interactions with RPG clearly dominate over electrostatic, and contribute to higher I_p and lower ΔE_p values compared to GC It is also clear from the results that favorable electrostatic and hydrophobic interactions with the surfactant at the surface can improve response at low ionic strength but are never as effective in producing good response as good probe-surface interactions observed at high ionic strength This indicates that at RPG the best (most sensitive) results will be obtained at the bare surface However reproducibility (and selectivity) will be a problem here and a small reduction in sensitivity is a worthwhile compromise if surface stability (and selectivity) is important. These results also show that unactivated GC even with effective surfactant modification will not be as sensitive as active RPG However, GC can become quite a sensitive surface in the absence of any other activation by simple addition of the right surfactant The advantage is a reasonably sensitive and selective continuously renewable surface The latter is a result of the dynamic surfactant-surface and probe-surfactant interactions

Furthermore, the results show that although catechol-surface interactions are significantly affected by ionic strength and electrolyte charge surfactant-surface and surfactant-probe interactions are not significantly affected indicating that surfactants serve as an effective ionic buffer The results presented here indicate that surfactant assembly at the surface is best at low ionic strength. These results also verify the importance of a favorable amphophilic environment for catechol response which can be provided by the surfactants when needed

Table 9 DOUBLE LAYER EFFECT ON SURFACTANT-RPG INTERACTIONS

SOLUTION[a]	[ELECTROLYTE][b] (mM)	I_p[c] (A/cm^{2}M)	ΔE_p[c] (mV)
CTAB/DOPAC	50	0.87 ± 0.06	39 ± 2
CTAB/DOPAC	500	0.83 ± 0.04	43 ± 4
CTAB/$MgCl_2$/DOPAC	55[d]	0.71 ± 0.01	43 ± 4
SDS/DA	50	0.86 ± 0.08	34 ± 5
SDS/DA	500	0.80 ± 0.05	43 ± 4
SDS/$MgCl_2$/DA	55[d]	0.74 ± 0.02	45 ± 3

[a] Probe concentrations were 0.50 mM with (3 mM) CTAB solutions containing DOPAC and (10 mM) SDS solutions containing DA

[b] Phosphate concentration at pH 7

[c] Oxidation peak currents normalized over electrode area (0.05 cm^2) and CV experiments were conducted at $v = 0.100$ Vs^{-1}

[d] $MgCl_2$ = 5 mM in addition to 50 mM phosphate at pH 7

CHAPTER 5
BILE SURFACTANT-MODIFIED GRAPHITE IN ELECTROCHEMICAL DETECTION OF PROTEINS

In this work we present a new strategy developed for the electrochemical detection of redox proteins at graphite surfaces The graphite electrode surface GC and RPG were chosen in this work for comparison due to their different activities where RPG is more active than GC Previously, it has been shown that GC is limited in analysis by poor sensitivity and RPG is limited by electrode fouling Ionic aliphatic surfactants used in surface modification were shown to solve these surface problems in the analysis of catecholamines A similar strategy is used in this work for the analysis of a redox protein cytochrome c (cyt c) However, a bulky, negatively charged biological surfactant deoxycholic acid (DCA) rather than an ionic aliphatic surfactant was used here for optimum surface and protein interactions preventing protein denaturing

DCA surfactant assembly used as modification strategy here prevents strong protein-surface interactions and irreversible protein adsorption extending the lifetime of the surface for analysis In addition, weak protein-modified (DCA) surface interactions are reversible and produce apparent formal potential ($E^{\circ\prime}$) values for cyt c near that of the native protein which is important in protein identification Another advantage of this modification scheme is an improvement in sensitivity of analytical signals Optimization of cyt c response was accomplished by adjusting parameters such as ionic strength and DCA concentration, and will be discussed later in detail as will be the factors determining the linear dynamic range and the sensitivity of this method An additional important outcome of this work is that the results obtained provide a clearer understanding of interactions necessary for sensitive as well as stable electrochemical response of different proteins at electrode surfaces including graphite and a clear model for graphite surface structure relevant in surface modification and the response of biological surface active molecules A detailed discussion of the interactions of cyt c at the bare and DCA-modified graphite surface as well as the quantitation of response in DCA solutions is provided here

5 1 Results and Discussion of Cyt c Analysis at Bare and Bile-Modified Graphite Electrode Surfaces

5.11 Protein-Bile Interactions and the Fundamentals of Electrochemistry of Cyt c at Graphite

At graphite good cyt c response consists of good sensitivity as a result of large surface concentration determined from large peak currents (i_p) and fast kinetics determined by small differences in anodic to cathodic peak potentials (ΔE_p) which indicate close surface access Improvements in the reusability of graphite by eliminating surface changes due to cyt c adsorption are also desired This can be done by preventing direct cyt c-graphite interactions

In order to accomplish this it is necessary to bring DCA effectively to the surface This can be done by increasing ionic strength and increasing DCA concentration. Assembly of DCA at surfaces in aqueous media is thought to occur through hydrophobic interactions with the surface (56,57). Increasing ionic strength decreases the compact double layer allowing closer access to the surface and improving hydrophobic interactions with the surface (12,13). Increasing DCA concentration increases the amount of DCA competing with cyt c for the surface, thus improving DCA assembly at the surface. Assembled DCA should effectively interact with cyt c, assuming proper orientation of DCA at the surface. If hydrophobic interactions with the surface drive DCA assembly then electrostatic interactions with cyt c can occur. In addition, chemical identification of cyt c is possible if the cyclic voltammetry (CV) measured $E^{\circ\prime}$ are consistent with that of native cyt c. This can be accomplished if cyt c-DCA interactions are weak and both redox forms interact with DCA equally (eq 9).

High ionic strength solutions produce a more compact double layer, typically allowing closer surface access for solution species (12,13). The high ionic strength, e.g. close surface access, favors hydrophobic interactions as indicated by previous results with catecholamines (chapter 4). If hydrophobic interactions between DCA and the surface drive assembly at both GC and RPG surfaces, these interactions should be best at high ionic strength. However, in order to maintain effective DCA-cyt c interactions a moderate ionic strength should be optimum since at ionic strength greater than 100 mM interactions between cyt c and DCA are weakened. The concentration of DCA in solution also affects cyt c response where high DCA concentrations leads to effective

DCA cyt c binding and a decrease in the amount of free cyt c in solution. This contributes to the effective competition by cyt c for the surface.

DCA is used to remove membrane bound cyt c through electrostatic attraction between DCA carboxylate groups and protonated amine groups of the amino acid lysine surrounding the exterior portion of cyt c (shown in Figure 9), at a pH where the carboxylic groups are deprotonated (between 8 and 10) (91). Twelve DCA molecules are reported to bind one cyt c molecule which correlates well with the net +13 charge of cyt c below pH 10 (91). Commonly, moderate ionic strengths of 100 mM are used in removing membrane bound cyt c (54,91). At pH ≥ 10 when cyt c is neutral, other interactions with DCA such as hydrogen bonding may occur which resemble bile-bile (DCA-DCA) interactions in secondary micelles (Figure 7) (91). In solution, cyt c interacts mainly with monomer surfactants and not aggregates (91). The interactions are weak and dynamic and can be investigated using electrochemical methods, e.g. by analyzing shifts in $E^{\circ\prime}$ (eq 9).

Because of the possibility of anion binding in solution, $E^{\circ\prime}$ of cyt c can be expected to be affected by the presence of different protein conformers other than the native form and by environmental parameters such as pH, ionic strength, the nature of the electrolyte, and temperature. However, at pH 8 where the carboxyl groups of DCA are deprotonated, no conformers of the native cyt c have been reported (92) and therefore, in the absence of other interactions no significant shifts in $E^{\circ\prime}$ should occur. Slight negative shifts in $E^{\circ\prime}$ for cyt c bound to anionic redox partners or to the cell membrane have been reported For example negative shifts of -0 045to -0 050 V have been observed for cyt c bound to cardiolipin-lecithin vesicles (86 93), negative shifts of -0 020 to -0 030 V for cyt c bound to phosvitin (94 95), -0 050 to -0 060 V negative shifts for mitochondrial membrane bound cyt c (96 97) and -0 035 to -0 040 V negative shifts for cyt c bound to cyt c oxidase or reductase in solution (98)

These shifts occur because the oxidized form of cyt c binds stronger to the anionic species than the reduced form (eq 9) and are not caused by protein unfolding since interactions are primarily electrostatic (weak) The magnitudes of the shifts are related to the ratio of the binding constants (eq 9) K_o/K_r which range between 2 (0 020 V shift) to 7 (-0 050 V shift) Negative shifts in $E^{\circ\prime}$ of -0 020 to 0 025 V for cyt c at tin oxide were observed by Bowden et al which were consistent with those reported for cyt c bound to phosvitin (25) This lead to their conclusion that interactions of cyt c at this surface were electrostatic

Large shifts in $E^{\circ\prime}$ can reflect irreversible conformational changes or the presence of other conformers (than the native form) in solution For example other conformers exist for native cyt c at alkaline pH (> 9) which exhibit large negative shifts in $E^{\circ\prime}$ (-0 42 V vs SCE) (99) compared to $E^{\circ\prime}$ of native cyt c at neutral pH (0 01 V vs SCE) However these conformational changes are not permanent and do not indicate protein unfolding (tertiary structure of the protein has not been destroyed) The conformers exhibit fast kinetics (small ΔE_p) confirming that there is no permanent change in the protein structure When protein tertiary structure is destroyed a large shift in E°, as well as a decrease in kinetics (large ΔE_p) are observed For example E° observed on Au were ca. 0.200 V more negative than of the native form and ΔE_p values were greater than 0 150 V indicating protein unfolding This is a result of the formation of a covalent bond between cyt c and Au (23)

DCA surface modification should produce a membrane-like structure at the surface If hydrophobic surface-DCA interactions occur (as predicted from earlier studies of DCA assembly Figure 7) orientation of DCA at graphite should leave carboxylate groups facing the solution which should favor cyt c-DCA interactions This orientation should be apparent from the results where an improvement in sensitivity e g larger I_p and smaller ΔE_p is expected if effective transport of cyt c to the surface by DCA occurs If weak electrostatic interactions between cyt c and DCA occur E° should be similar to native membrane-bound cyt c with a possible small negative shift relative to free solution form and because of the differences in DCA interactions with oxidized and reduced forms of the protein ΔE_p values reflecting fast kinetics

5.12 Cyt c Response at Bare and Bile-modified GC

At GC Bond et al observed two well-defined reductions of cyt c (22) The two pathways gave rise to two different E° values One for irreversibly adsorbed cyt c conformer at 0 42 V vs SCE and the other for native cyt c in solution at 0 01 V vs SCE The irreversibly adsorbed cyt c had a large ΔE_p value consistent with permanent conformational changes On the other hand ΔE_p values for native cyt c indicated quasireversible kinetics Furthermore, since the adsorption process for cyt c was observed in the absence of modifiers at GC eventual loss of signal can be expected but was not reported

The results in Table 10 show an increase in I_p and a decrease in ΔE_p for cyt c at GC following DCA addition The E°' measured from CV for cyt c at bare GC (Table 10) indicate slight negative shifts in E°' compared to E°' of the native cyt c in solution and are consistent with E°' reported for cyt c bound to anionic redox partners such as cyt c oxidase and reductase (0 035to -0 040 V vs SCE) (98) From these E°' values a binding constant ratio (K_{ox}/K_{red}) of 4 (eq 9) was calculated at GC again, consistent with that reported for cyt c electrostatically interacting with cyt c oxidase or cyt c reductase (98)

Since following DCA addition shifts in E°' for cyt c are small at GC, this indicates that DCA-cyt c binding is primarily electrostatic and does resemble that of cyt c bound to cyt c oxidase/reductase (-0 040 V shift in E°' from native form) (98)

The CV shown in Figure 15 (A) indicates that in the absence of DCA surface changes occur at GC with increasing number of CV scans as is evident from the decrease in I_p However, following DCA addition (Figure 15 (B)) the response for cyt c becomes stable and more sensitive As predicted DCA assembly is effective in reducing surface changes as well as enhancing response for cyt c at GC

Table 10. RESPONSE OF CYTOCHROME C AT BARE AND DCA MODIFIED GRAPHITE[a]

SOLUTION	SURFACE[b]	I_{red}[c] (μA)	ΔE_p (mV)	$E_{1/2}$ (mV)
CYT C	GC	0.80 ± 0.05	107 ± 2	-27 ± 2
CYT C/DCA[d]	GC	1.05 ± 0.05	66 ± 4	29 ± 1
CYT C	RPG	0.60 ± 0.07	63 ± 6	-43 ± 1
CYT C/DCA[d]	RPG	0.90 ± 0.03	55 ± 0	-32 ± 2

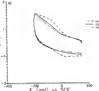
[a] CV results at $v = 0.100$ Vs^{-1}. All solutions contain 110 μM cyt c and 100 mM phosphate buffer at pH 8

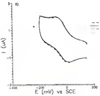
[b] Electrode areas of GC = 0.06 cm^2, RPG = 0.05 cm^2

[c] Background subtracted reduction peak currents.

[d] Same as [a] with 2 mM DCA added

Figure 15 CV of Cyt c (100 μM) at GC as a Function of Scan Number (A) bare GC (B) DCA-Modified GC (1 mM DCA) v = 0.100 V^{-1} and electrode area = 0.06 cm^2 in 100 mM phosphate buffer at pH 8


A)
3
1
-1
-3
-400
-200
0
200
I (uA)
E (mV) vs SCE
run 1
run 2
run 3


B)
3
1
-1
-3
-400
-200
0
200
I (uA)
E (mV) vs SCE
run 1
run 2
run 3

5 13 Cyt c Response at Bare and Bile Modified RPG

The response of cyt c at RPG in the absence of modification was expected to be good due to the greater activity of RPG than GC However irreversible surface interactions may be a greater problem here than at GC since more hydrophobic areas are exposed at this surface Similar to what was observed at GC DCA improved cyt c response at RPG as evident from the increase in i_p and decrease in ΔE_p following DCA addition (Table 10) This suggests that DCA assembly and DCA-cyt c interactions are similar at both surfaces However the inherent inactivity of GC allows modification with DCA to have a more dramatic effect on response than at RPG where cyt c response is good in the absence of DCA

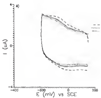
The $E^{\circ\prime}$ values measured by CV at bare RPG (Table 10) show a slight negative shift in $E^{\circ\prime}$ compared to $E^{\circ\prime}$ of native cyt c in solution and are consistent with those expected for cyt c bound to anionic groups at a membrane (-0 050 to -0 060 V vs SCE) (96 97) The E° values at bare RPG are slightly more negative than those at bare GC indicating that the binding of the oxidized form of cyt c is stronger at RPG than at GC (eq 9) The K_{ox}/K_{red} ratio at bare RPG was determined to be 7 (eq 9) consistent with that reported for membrane-bound cyt c. This large K_{ox}/K_{red} is attributed to primarily electrostatic interactions of cyt c with the surface with some hydrophobic contribution (96 97) On the other hand, at GC primarily electrostatic interactions occur and the ratio of K_o/K_r is smaller The importance of electrostatic and hydrophobic effects at this surface is supported by these results

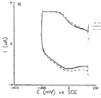
At RPG there is a positive shift in $E^{\circ\prime}$ from that observed at the bare surface following DCA addition The positive shift in $E^{\circ\prime}$ confirms that DCA assembles at the surface resulting in weaker binding between the oxidized form of cyt c relative to that at the bare surface In addition a new surface environment is created in the presence of DCA. The increase in $E^{\circ\prime}$ in DCA relative to the bare surface indicates a decrease in hydrophobic and an increase in electrostatic environment for cyt c and confirms the similarity in modified surface environment at GC and RPG

The results in Figure 16 indicate that in the absence of DCA surface changes occur at RPG with scan repetition Similarly to what was observed at GC this results in a decrease in I_p. However following DCA addition the response becomes stable

These results indicate that DCA modification of GC and RPG is successful in increasing the lifetime as well as the sensitivity of these surfaces while maintaining the native structure of cyt c The improvement in surface environment is similar at both surfaces, although more dramatic effects are observed at GC than RPG This is attributed to differences in activities between these two surfaces. Inactive GC is more sensitive to surface changes resulting in large changes in surface activity with minimal modification while active (heterogenous) RPG is less sensitive to surface changes

Figure 16 CV of Cyt c (100 μM) at RPG as a Function of Scan Number: (A) bare RPG (B) DCA Modified RPG (1 mM DCA). $v = 0.100$ Vs^{-1} and electrode area = 0.05 cm^2 in 100 mM phosphate buffer at pH 8


A)
4
0
-4
I (uA)
-400
-200
0
200
E (mV) vs SCE
run 1
run 2
run 3


B)
4
0
-4
I (uA)
-400
-200
0
200
E (mV) vs SCE
run 1
run 2
run 3

5 2 Surface Model for Bile Assembly at Graphite Electrode Surfaces

To verify the kinds of DCA interactions which occur with graphite surfaces the response of dopamine (DA) at DCA-modified graphite was also investigated DA is positively charged at neutral pH and has both hydrophobic and hydrophilic properties causing it to interact with graphite and expectedly with DCA In addition DA size and known electrochemical response allows it to be used as a probe to obtain a clear model for DCA assembly at graphite Earlier it was shown that DA and GC interactions were governed by electrostatic effects At RPG the combination of electrostatic attraction and hydrophobic interactions resulted in irreversible adsorption and concomitant surface changes In an attempt to separate the effect of DCA-graphite and DCA DA interactions GC and RPG were chosen because of their different interactions with DA

The assembly of DCA should compete with DA-surface interactions and should provide a favorable amphophilic environment at the surface for DA response, assuming the assembly leaves the carboxylate groups facing the solution (Figure 7) which will allow electrostatic interactions with positively charged DA Favorable DCA DA interactions should enhance the sensitivity of GC and reduce surface changes at both surfaces without sacrificing the sensitivity of RPG

As with cyt c, ionic strength will affect DCA-surface and DCA-DA interactions Here high ionic strength should be best because both surface and probe interactions with DCA are heavily governed by hydrophobic effects The results presented here were obtained in 0.50 M phosphate buffer compared to 0.10 M used in cyt c analysis. When low ionic strength phosphate buffers were used at GC, the effect of DCA on DA response was not apparent, indicating no significant DCA-DA interactions consistent with hydrophobic interactions controlling assembly. At RPG, DCA assembly was insensitive to ionic strength presumably because of the greater inherent hydrophobicity of RPG.

These results differ from those with cyt c, where DCA was most effective at both surfaces at low ionic strength. There are two interactions to consider here. First, the interactions of probe with DCA. Then, the interactions of DCA with the surface. DCA interactions with the surface are best at high ionic strength. However, DCA-cyt c interactions are best at lower ionic strength. Apparently, the latter is more important for effective improvement in cyt c response and, therefore, a compromise in ionic strength is necessary. On the other hand, in DA analysis high ionic strength appears to favor both DCA-probe and DCA-surface interactions.

Similar to the effect of aliphatic surfactants, DCA should affect the response of DA differently at GC and RPG due to different probe-surface interactions. Apparently, the presence of DCA at the surface increases DA-RPG distance without changing the amount of DA the surface. This is consistent with electrostatic interaction between DA and DCA being favorable resulting in no significant change in surface concentration of DA. The results presented in Table 11 indicate that DA kinetics at GC improve in DCA solutions as evident

Table 11 DA RESPONSE AT BARE AND DCA MODIFIED GRAPHITE

SOLUTION	SURFACE[b]	ΔE_p (mV)	i_{peak}[c] (μA)	m[d]
DA	GC[f]	169 ± 29	8 ± 2	--------
DA/DCA[e]	GC	116 ± 31	9 ± 1	--------
DA	RPG[g]	49 ± 5	17 ± 1	0.74 ± 0.14
DA/DCA[e]	RPG	64 ± 1	16 ± 2	0.62 ± 0.04

[a] CV results with $v = 0.100\ Vs^{-1}$ All solutions contain 0.5 mM DA and 0.50 M phosphate buffer at pH 8

[b] Electrode areas GC = 0.06 cm^2 RPG = 0.05 cm^2

[c] Background subtracted oxidation peak currents

[d] Slopes of log i_p vs log v with v ranging between 0.10 and 1.0 Vs^{-1}

[e] Same as [a] with 5 mM bile added

[f] $i_{p,irrev}$(theoretical) = 10 uA, with $D_0 = 6.0 \times 10^{-6}\ cm^2s^{-1}$ (eq 11)

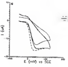
[g] $i_{p,rev}$(theoretical) = 15 uA (eq 10)

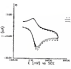
from the decrease in ΔE_p which indicates closer surface access At RPG ΔE_p (Table 11) increases in DCA solutions indicating greater surface distance This is presumably due to a decrease in DA adsorption as evident from the decrease in the slope of the log i_p vs log v

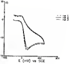
Values of i_p for DA in Table 11 do not change at either surface indicating that DCA has no significant effect on the surface concentration No effective preconcentration at either surface occurs presumably because of the proposed monolayer assembly of DCA at the surface consistent with that reported by Ekwall et al. (56 57) Primary and secondary micelles present in solution at these DCA concentrations can interact with DA electrostatically and hydrophobically, while the monolayer assembly at the surface provides a primarily favorable hydrophilic environment with little hydrophobic advantage The greater i_p for DA at RPG over GC is a result of the inherent differences in electrochemical activities of each surface At RPG DA response in DCA remains reversible, where at GC the response is still irreversible Under the experimental conditions, theoretical i_p for a reversible two electron diffusion controlled system (eq 10) is 15 µA, while for an irreversible system (eq 11) it is 10 µA These values are in agreement with the observed values in Table 11

In previous work with aliphatic surfactants conducted at pH 7 RPG but not GC suffered from surface changes with scan number The present experiments were conducted at pH 8 At this pH both surfaces were susceptible to surface changes with scan number as evident from the results in Figure 17

Figure 17 CV of DA (0.50 mM) at Graphite Surfaces as a Function of Scan Number. (A) bare GC. (B) bare RPG (C) DCA-modified GC (5 mM DCA) as a function of scans with $v = 0.100\ \mathrm{Vs^{-1}}$ and electrode area $= 0.06\ \mathrm{cm^2}$ and $0.05\ \mathrm{cm^2}$ for GC and RPG, respectively, in 0.05 M phosphate buffer at pH 8


A)
10
0
-10
-20
I (uA)
-400
0
400
800
E (mV) vs SCE


B)
10.00
-10.00
-30.00
I (uA)
-200.00
0.00
200.00
E (mV) vs SCE


C)
10
0
-10
-20
I (uA)
-400
0
400
800
E (mV) vs SCE

and previous results with cyt c. Based on these results it appears that the extent of surface changes depends on pH, surface activity and potential window used in analysis. Increasing solution pH can change the functional groups present at the surface and increases hydroxide concentration in solution which can result in easier surface oxidation. RPG is an active surface, more susceptible to changes than GC. Problems with surface changes of GC at pH 8 were overcome by DCA addition, as shown in Figure 17. However, no significant improvement in surface changes at RPG was observed following DCA addition.

Unlike the results for cyt c where the surface stability is improved at both surfaces (Figures 15 and 16), DCA did not improve stability of RPG for DA analysis, presumably because of the different potential windows used. For DA oxidation, a more positive potential window (0.0 to 0.400 V) is needed than for reduction of cyt c (0.100 to -0.200 V). This must result in greater susceptibility to surface oxidation (surface changes) for RPG.

The results for DA and cyt c at DCA-modified graphite indicate that DCA assembles at graphite through its hydrophobic side, allowing favorable electrostatic interactions to occur with DA and cyt c. The enhancement in response is more dramatic for cyt c due to the effective electrostatic cyt c-DCA interactions. Electrostatic cyt c-DCA interactions occur primarily with monomers in solution while DA-DCA interactions are due to a combination of hydrophobic and electrostatic interactions which are favored with the larger aggregates present in solution over the monolayer surface assembly. The presence of a monolayer assembly at the surface does not provide a significantly hydrophobic environment needed to see a dramatic improvement in DA response However, the decrease in DA GC distance and decrease in DA adsorption at RPG observed following DCA addition does confirm DCA assembly at the surface with the predicted orientation

DCA-modification of graphite appears to be similar to modification of Au with 4 4-bipyridyl (4-bpy) (100-102) Surface assembly of both is a competitive process where cyt c is competing with the modifier for the surface Interactions at the surface are optimum at the optimum DCA cyt c solution ratio High 4-bpy concentrations are necessary to significantly reduce the presence of irreversibly adsorbed form of cyt c at Au and a 12 1 DCA cyt c ratio is best for most sensitive response at DCA-modified surface

However, there are significant differences between the two modification schemes DCA appears to compete more effectively than 4-bpy with cyt c for the surface because of its amphophilic properties and there is no evidence of cyt c desorbing DCA with time. However, interactions between 4-bpy and Au are weaker than those between cyt c and Au resulting in easy replacement of 4-bpy at the surface by cyt c (101 102) In fact, at low 4-bpy concentrations the response for the adsorbed irreversible form of cyt c at Au is observed Even at high 4-bpy concentrations it was found that the structure of cyt c co-adsorbed with 4-bpy at Au was significantly different from that of any (free or bound) native form of cyt c (101,102) DCA modification of graphite appears to be much more effective in maintaining native cyt c structure than 4-bpy modification of Au In fact the response for cyt c observed at DCA-modified graphite as measured by shifts in $E^{\circ\prime}$ reflects weak electrostatic interactions of cyt c in vivo bound to cyt c oxidase or reductase

5 3 Quantitation and Optimization of Response at Bile-Modified Graphite Electrodes

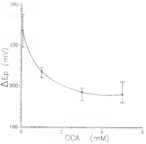
5 31 Quantitation of Catecholamine Response in Bile Surfactant Solutions

To determine the optimum DCA concentration for DA response, the response of DA as a function of DCA concentration was measured at GC An increase in DCA DA interactions was expected to improve DA response only if the behavior changed from irreversible to reversible GC was chosen because the probe response at this inactive surface is sensitive to microenvironment changes

Since DCA did not affect DA i_p but did affect ΔE_p, ΔE_p values were used to determine optimum DCA concentration for DA analysis A decrease in ΔE_p could translate into improved sensitivity if the behavior changes from irreversible at the bare surface to reversible in the presence of DCA. From Figure 18, ΔE_p values are shown to decrease with DCA concentration. However, ΔE_p still remains in the irreversible regime which accounts for the observed small changes in i_p and the lack of improvement in sensitivity (Table 12)

In Figure 18, a decrease in ΔE_p is observed until cmc of DCA (ca. 3 mM) was reached This corresponds to a concentration ratio of 6 1 of DCA DA in solution

Figure 18 DA Response as a Function of DCA Concentration ΔE_p from CV of 0.50 mM DA in 0.50 M phosphate buffer at pH 8 at GC with the $v = 0.100\ \mathrm{Vs}^{-1}$ and electrode area = 0.06 cm^2


240
220
200
180
0
2
4
6
ΔEp (mV)
DCA (mM)

As shown in Figure 19 the response is linear (correlation coefficient of 0 999) over the concentration range between 60 1 and 1 1 DCA DA. The results in Table 12 show that in this concentration range the sensitivity of DCA-modified GC as measured from the slope of the calibration plot does not improve over that measured at the bare surface However, the sensitivity is greater than the theoretical sensitivity for a two electron irreversible diffusion controlled system (eq 11) This is consistent with the observed preferential interactions of cations at this surface. Most importantly, in the presence of DCA the surface remains stable

The intercept of 1 5 μA for the working curve in Figure 19 represents background currents (e g , charging currents) since the results were not background corrected This indicates that for a signal to noise ratio of 3 1 the lowest currents detectable are 4 6 μA corresponding to a limit of detection (LOD) of 70 μM DA At DA concentrations lower than those used in Figure 19 (less than 0 05 mM) similar sensitivity is expected since the sensitivity is independent from DCA DA concentration ratio in this concentration range When aggregates exist in solution (at DCA concentrations greater than cmc), DCA DA binding constants are fixed and surface interactions are best Therefore by working at cmc both maximum sensitivity and stability can be achieved

5 32 Quantitation of Cyt c Response in Bile Surfactant Solutions

The optimum conditions for cyt c analysis were also determined. Since cyt c interacts with DCA in solution in a ratio of 12:1, DCA.cyt c (91), this ratio

Table 12 SENSITIVITY OF DCA MODIFIED GC

PROBE	LDR	SENSITIVITY[a] BARE GC (A/Mcm^2)	SENSITIVITY DCA-GC (A/Mcm^2)	THEORETICAL SENSITIVITY	LOD[b] (μm)
DA	≈ 3	0.46	0.46	0.33[c]	70
CYT C	≈ 1	0.12	0.21	1.7×10^{-6} [d]	9

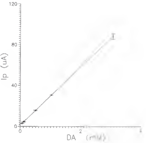
[a] Sensitivity determined from the calibration plots in Figures 20 and 21. Correlation coefficients of 0.999 and 1.00 respectively. Electrode area 0.06 cm^2. $v = 0.100\ Vs^{-1}$

[b] Limit of detection for DCA-modified GC

[c] Theoretical sensitivity (A/cm^{2}M) for an irreversible diffusion controlled system (eq 11) with $\alpha = 0.5$, $n = 2$, $n_a = 1$, $A = 0.06\ cm^2$, DA = 0.50 mM and $D_o = 6.0 \times 10^{-6}\ cm^2s^{-1}$ (eq 11)

[d] Theoretical sensitivity (A/cm^2)for a reversible adsorption controlled system (eq 11) with n= 1, $\Gamma = 18.56\ pmolcm^{-2}$ (calculated using area under a CV curve for a 23 μM cyt c solution), and $v = 0.100\ Vs^{-1}$ (eq 12)

Figure 19 DA Response at Optimum DCA Concentration (3 mM) at DCA-Modified GC. CV (i_p) of DA at concentrations between 0.05 and 3 mM in 0.50 M phosphate buffer at pH 8 at GC with $v = 0.100$ Vs^{-1} and electrode area = 0.06 cm^2

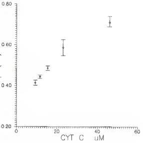

120
80
40
0
Ip (uA)
0
2
4
DA (mM)

was expected to yield the best sensitivity. In addition to interactions in solution, the effect of DCA concentration on the interactions with the surface must be considered in quantitating cyt c response. We observed a linear increase in response of cyt c with correlation coefficient of 0.998 with increasing DCA concentration up to 0.50 mM. Eventually a decrease in response was observed after the ratio of 12:1 DCA:cyt c is reached. The 12:1 DCA:cyt c molar ratio must, therefore, be most effective not only for DCA:cyt c solution but also for DCA-cyt c surface interactions.

At the optimum DCA:cyt c ratio, a linear dynamic range was also determined. The response at 0.50 mM DCA was linear for cyt c concentrations between 9 and 23 μM as shown in Figure 20. Beyond 23 μM cyt c response plateaus. The 23 μM concentration of cyt c corresponds to a surface coverage of 18 pmol/cm². This coverage is consistent with the model for DCA-cyt c interactions at the surface discussed earlier in this paper where cyt c is electrostatically adsorbed at DCA-modified GC by interactions between the positively charge lysine groups of cyt c and the carboxylate groups of DCA. Similar surface coverages (between 15 and 20 pmol/cm²) for adsorbed cyt c at a self-assembled carboxyl-terminated alkanethiolate monolayers with alkyl chain length ranging between 6 and 16 carbons on Au have been reported by Bowden et al. (99).

Assuming the cyt c heme group is facing the DCA-modified electrode

Figure 20 Cyt c CV Response at Optimum DCA Concentration (0.50 mM). CV (i_p) of cyt c at concentrations between 9 and 46 μM in 35 mM phosphate buffer at pH 8 at GC with $v = 0.100\ Vs^{-1}$ and electrode area = 0.06 cm^2


0.80
0.60
0.40
0.20
Ip (uA)
0
20
40
60
CYT C uM

surface during cyt c adsorption facilitating electron transfer, the theoretical value for the number of cyt c molecules necessary for monolayer coverage is 7 09 x 10^{11} molecules This corresponds to a projected area of 8 75 X 10^{-14} cm^2 per cyt c molecule that was calculated using the dimensions of cyt c 25 by 35 Å, as shown in Figure 8 Experimentally determined surface coverage is 6 85 x 10^{11} molecules of cyt c which is close to the theoretical value confirming that the heme group faces the modified surface Kinetics for cyt c at DCA modified GC are fast confirming favorable orientation of the heme group for electron transfer

Table 12 shows that the sensitivity of the method for cyt c detection calculated from the slope of the calibration plot is greater than at the bare surface The theoretical i_p for an adsorption controlled reversible process (eq 12) with the surface coverage of 18 56 pmol/cm^2 is 1 7$_8$ x 10^{-6} A/cm^2 while the measured experimental i_p for the 23 µM solution is (1 7$_3$ ± 0 01) x 10^{-6} A/cm^2 The agreement between experimental and theoretical values is consistent with a monolayer coverage of cyt c at the surface and the reversible adsorption controlled process

The linear dynamic range for cyt c was calculated by diluting the original cyt c solution while maintaining the DCA cyt c ratio of 12 1 Dilutions resulted in a maximum 5 fold change in ionic strength of the DCA.cyt c solution Because of the desalting methods used in cyt c purification the ionic strength was only known approximately and was not adjusted in the measurements The initial ionic strength was low at a ca. 35 mM phosphate concentration. In spite of the changes in ionic strength, linearity in cyt c response was observed in the concentration range from 9 to 48 μM, indicating that background currents and signal to noise ratios had a small effect on response in this experiment, e.g. changes in ionic strength had little effect on response. Following dilution of the original cyt c solution, however, some improvement in response for cyt c was observed where the background currents appeared to significantly decrease. A poor response (signal barely observed over background currents) was observed for a 9 μM solution of cyt c indicating that detection of lower concentrations would not be feasible. Therefore, the LOD was estimated to be ca. 9 μM.

5.4 Conclusions

The results confirm that at DCA-modified graphite the response for cyt c is due to electrostatic adsorption of cyt c at the DCA assembly. As expected, DCA adsorption occurs through hydrophobic interactions which allows the carboxylate groups to face the solution and interact (electrostatically) weakly with cyt c. This is confirmed by fast electron transfer of cyt c at DCA-modified surface and small negative shifts in $E^{\circ\prime}$ compared to the native solution form. Therefore, DCA-modification of graphite maintains the inherent good properties of graphite for bioanalysis while improving surface stability and sensitivity. Quantitation of cyt c response in DCA media provided an optimum ratio of 12:1 DCA:cyt c for which an improvement in sensitivity over bare surface and that predicted theoretically was observed.

In the analysis of DA, aliphatic surfactant modification was shown to improve sensitivity of inactive GC and reusability of active RPG without sacrificing its sensitivity (76 103) DCA modification did not have a significant effect on RPG response of DA, although at GC a more stable surface was created DCA assembly was not effective in preconcentrating DA, presumably because interactions with solution aggregates were favored over DA-modified surface interactions At the higher pH needed for DCA dissociation and at the positive potential window for DA analysis, this surfactant is not as effective in preventing surface changes at active RPG The small decrease in DA response at RPG in DCA solutions is consistent with a decrease in adsorption Quantitation of response for DA in DCA solution indicated that the presence of larger aggregates was best for good DA response Therefore, an enhancement in sensitivity was not observed over the bare surface due to only monolayer formation of DCA at the surface However, an improvement in surface access was observed at the DCA-modified surface

CHAPTER 6
IONIC ALIPHATIC SURFACTANT-MODIFIED CARBON FIBER DISK ELECTRODES IN ELECTROCHEMICAL DETECTION OF CATECHOLAMINES

Ultramicroelectrodes have many advantages over conventional size electrodes in bioanalysis such as better detection limits and more rapid response time. In addition, their small ohmic potential (iR) drop in solution allows analysis to be conducted in organic media or at low ionic strength. Unfortunately, at untreated carbon fiber electrodes the electrochemistry of catecholamines is slow and peaks are very poorly defined (104,105). Similarly to what was observed at GC, preferential interactions with positively charged species such as dopamine (DA) have been observed (14-16). Unlike at GC, DA adsorption at the untreated carbon fiber has been reported (16,104,105). The response for negatively charged DOPAC is even slower and more poorly defined than for positively charged DA with no evidence of adsorption.

Pretreatment is an important factor in the sensitivity of response at carbon fiber electrodes. Adams et al. showed how the extent of anodic pretreatment affects response at carbon fiber (104). Strong (0 to 2-3 V) and mild (0.0 to 1.3-2.0 V) electrochemical pretreatment (ECP) methods have been employed to improve the activity of carbon fibers in the analysis of biological molecules such as catecholamines. Following mild ECP, an increase in DA adsorption and a decrease in DOPAC response was observed Strong ECP was found ideal for in vivo analysis of DA, since this pretreatment enhanced response for DA and decreased response for negatively charged ascorbic acid (AA) which usually interferes with DA analysis (104) It was found that ECP at potentials greater than 3 V results in a decrease in response for all catecholamines presumably because the surface has been extensively oxidized This forms an insulating layer at the surface which partially covers the surface The small response observed is due to the small percent of uncovered cracks (basal plane regions) at the surface which allow access to all catecholamines and do not discriminate based on charge Surface fouling as a result of surface oxidation was also observed at slow scan rates and at positive potential scans (3)

ECP methods only improve response for positively charged catecholamines This improvement in response is a result of increased adsorption at the surface which in turn results in problems with surface reusability (104) The focus of this work is to apply a similar strategies used in modification of conventional size graphite electrodes to the modification of carbon fiber disk electrodes thereby enhancing their properties for biological analysis

Surfactant modification of the carbon fiber surfaces should provide a simple method to increase the sensitivity of carbon fibers for bioanalysis while enhancing the lifetime of the electrode by reducing surface changes such as surface oxidation In addition, the presence of surfactant in solution is expected to result in a dynamic continuous exchange between surfactant in solution and at the surface, providing a continuously renewed surface free of memory effects similar to that observed for conventional size electrodes A detailed discussion of the response of catecholamines at bare and surfactant-modified carbon fiber disk electrodes as well as the parameters such as ionic strength needed for optimum response will follow

6.1 Results and Discussion for Catecholamine Response at Bare Carbon Fiber Surfaces

The results in Table 13 indicate that DA exhibits a better response than DOPAC at this surface at low ionic strength The plateau potential (E_p) for DA is better defined than for DOPAC where no plateau is observed These results are consistent with preferential cation interactions previously reported at this surface (16,104 105) In addition, the limiting current (i_l) for DA is much greater than the theoretical value of 0 00530 A/cmM (eq 14) confirming surface preconcentration

The response of catecholamines at this surface is similar to that at GC where preferential cation interactions is observed and electrostatic effects are important Therefore, it was expected that anionic probe-surface interactions (DOPAC) should improve at this surface with increasing ionic strength while the opposite was expected for cationic probes (DA). Control of ionic strength was expected to enable manipulation of catecholamine response at this surface

The results in Table 13 show that at higher ionic strength DOPAC-carbon fiber interactions improve since a plateau is observed As expected, an increase in ionic strength decreases DA response at carbon fiber disk electrode as evident

Table 13 RESPONSE OF CATECHOLAMINES AT A CARBON FIBER DISK ELECTRODE

PROBE[a]	[PHOSPHATE][b] (M)	I_l/Cr[c] (A/Mcm)	E_{pc}[d] (mV)
DOPAC	0.050	NO PLATEAU	————
DOPAC	0.50	0.00482 ± 0.00002	406 ± 12
DA	0.050	0.01468 ± 0.00005	253 ± 7
DA	0.50	0.00487 ± 0.00003	393 ± 9

[a] Probe concentrations (C) = 5 mM

[b] Phosphate buffer at pH 7

[c] Limiting oxidation currents normalized over electrode radius (r) = 2.7 ± 0.7 μm and probe concentration of 5 mM

[d] Oxidation peak plateau measured at v = 0.010 Vs^{-1}

from the decrease in i_l and a positive shift in E_p, indicating lower surface concentration and slower kinetics. In addition, the response for DOPAC and DA becomes comparable at high ionic strength, e.g., similar i_l and E_p with i_l approaching theoretical values for this electrode radius (eq 14). These results indicate that at high ionic strength when surface groups are screened interactions with the surface are improved, resulting in a similar probe response for DOPAC and DA. In addition, at high ionic strength the surface resembles RPG since both probes can access the surface similarly. These results suggest that at low ionic strength, when the surface groups are exposed, electrostatic interactions with the surface dominate. However, at high ionic strength when the surface groups are screened, electrostatic interactions are weak resulting in similar interactions for catecholamines independent of charge.

6.2 Catecholamine Response at Aliphatic Surfactant-Modified Carbon Fiber Surface

6.2.1 Ionic Strength Effects on Aliphatic Surfactant-Surface Interactions at Carbon Fiber

The effect of ionic strength on aliphatic surfactant-surface interactions at conventional size electrodes (GC and RPG) was found to be less dramatic than for probe-surface interactions. In addition, aliphatic surfactant assembly at active RPG appeared to be insensitive to ionic strength effects. At carbon fiber disk electrodes, electrostatic interactions appear to be important to the response of catecholamines. The results in Table 14 indicate that cetyltrimethylammonium bromide (CTAB) -surface interactions are best at low ionic strength as evident from the greater enhancement in response of DOPAC relative to the bare surface (Tables 13 and 14) where a greater i_l and lower E_p is observed at low ionic strength while at high ionic strength CTAB addition has no significant effect on DOPAC response compared to the bare surface (Table 13). On the other hand, sodium dodecyl sulfate (SDS)-surface interactions are best at high ionic strength since DA response at high ionic strength improves in the presence of SDS (Table 14) over the bare surface (Table 13), while at low ionic strength DA response is poor in SDS solution (Table 14) and is much better at the bare surface (Table 13).

Ionic strength effects on CTAB and SDS surfactant assembly at the carbon fiber surface suggest that electrostatic interactions drive surfactant assembly here, similar to what was observed at GC and RPG. Surfactant-surface interactions at carbon fiber disk electrodes are more susceptible to changes in ionic strength than at GC or RPG, consistent with the greater hydrophilicity of this surface due to the presence of an excess of edge planes (4). Earlier it was shown that aliphatic surfactant-RPG interactions were insensitive to ionic strength since probe response did not change (Table 7). In addition, although aliphatic surfactant-GC interactions did change with increasing ionic strength (Table 6), the change was less dramatic than observed for carbon fiber electrodes (Table 14).

6.22 Surfactant Effect on Surface Stability

In addition to improving probe response, e.g. peak plateau, aliphatic

Table 14 SURFACTANT EFFECT ON CATECHOLAMINE RESPONSE AT A CARBON FIBER DISK ELECTRODE

PROBE[a]	[PHOSPHATE][b] (M)	i_l/Cr[c] (A/Mcm)	E_{pc}[d] (V)
DOPAC/CTAB	0.050	0.00467 ± 0.00002	336 ±15
DOPAC/CTAB	0.50	0.00502 ± 0.00003	375 ± 10
DA/SDS	0.050	NO PLATEAU	————
DA/SDS	0.50	0.00930 ± 0.00006	300 ± 8

[a] Probe concentration (C) = 5 mM, DOPAC response in 3 mM CTAB solutions and DA response in 10 mM SDS solutions

[b] Phosphate buffer at pH 7

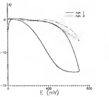
[c] Limiting oxidation currents normalized over electrode radius (r) = 2.7 ± 0.7 μm and probe concentration of 5 mM

[d] Oxidation plateau potential measured at $v = 0.010$ Vs^{-1}

surfactants in solution should improve surface reusability similarly to what was observed at GC and RPG The results in Figure 21 show response of DOPAC at bare and CTAB modified carbon fiber disk electrodes at 0 50M This ionic strength was chosen because at low ionic strength no plateau for DOPAC is observed at the bare surface This figure indicates significant surface changes with scan repetition in the absence of CTAB (Figure 21 (A)) Following CTAB addition the response becomes stable with scan number (Figure 21 (B)) A similar but less dramatic effect was observed for DA following SDS addition The improvement in surface stability is less dramatic in SDS solutions, presumably, due to the favored interactions of CTAB over SDS with the surface resulting in a better assembly that is more capable of preventing surface changes

In addition to surface changes resulting from probe adsorption, surface changes as a result of surface oxidation shorter time spent at oxidizing potentials must be considered at graphite surfaces such as carbon fiber Surface changes during analysis were observed to decrease with increasing scan rate (v) Apparently, at faster v the surface is less susceptible to surface oxidation and concomitant surface changes This is evident from the comparison of Figures 21 and 22 run at 0 010 and 0.100 Vs^{-1}, respectively These figures show that the surface is more susceptible to surface changes at lower v Furthermore, from Figure 22 it is apparent that even at faster v surfactants provided an additional enhancement in surface stability with better response, e g better defined plateau and less positive E_p than observed at the bare surface Again, a similar but less

Figure 21 CV for DOPAC at a Carbon Fiber Electrode $v = 0.010\ Vs^{-1}$, r = of 2.7 µm, and C = 5 mM DOPAC in 0.50 M pH 7 phosphate buffer. Currents reported have been amplified 10^4 times
a) bare surface, b) CTAB modified surface (with 3 mM CTAB)


A)
run 1
run 3
0
-10
-20
I (uA)
0
400
800
E (mV)

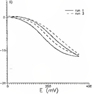
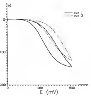
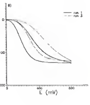

B)
run 1
run 3
0
-10
-20
I (uA)
0
200
400
E (mV)

Figure 22 Scan Rate Effects on Surface Stability. CV for DOPAC at carbon fiber at $v = 0.100\ Vs^{-1}$ (A) bare (B) CTAB-modified (3 mM CTAB). As a function of scan number with $r = 2.7\ \mu m$, and $C = 5$ mM DOPAC in 0.50 M pH 7 phosphate buffer. Currents have been amplified by 10^4


A)
run 1
run 3
I (uA)
0
-100
-200
0
400
800
E (mV)


B)
run 1
run 3
I (uA)
0
-100
-200
0
400
800
E (mV)

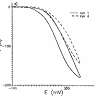
dramatic effect was observed for DA, where response at the bare and SDS modified surface was more stable at faster v. Figure 23 shows the greater stability of response for DA at SDS-modified carbon fiber electrode run at a faster v of 0.100 V^{-1} than at 0.010 Vs^{-1}

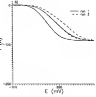
6.3 CONCLUSIONS

From these results it is clear that aliphatic surfactant assembly improves the stability of the carbon fiber disk electrode for catecholamine analysis, primarily for anionic catecholamine, DOPAC, which otherwise exhibits poor response at the unmodified surface. This can be expected since the carbon fiber surface resembles GC where electrostatic interactions are important. The carbon fiber surface required no pretreatment to observed the improved response in aliphatic surfactant solutions. Before surfactant assembly the fiber was simply polished with gamal alumina.

Similar to the results at GC and RPG, cationic surfactant assembly appears to be more effective at the carbon fiber surface, consistent with preferential cationic interactions with the surface. In addition to the use of surfactants, surface changes during analysis could also be reduced by working at faster v thereby limiting surface oxidation.

Figure 23 Scan Rate Effects on Surface Stability. CV for DA at SDS-modified carbon fiber at (A) $\nu = 0.010\ \mathrm{Vs}^{-1}$, (B) $\nu = 0.100\ \mathrm{Vs}^{-1}$. As a function of scan number with $r = 2.7\ \mu\mathrm{m}$ and $C = 5$ mM DA and 10 mM SDS in 0.50 M pH 7 phosphate buffer. Currents have been amplified by 10^4


A)
run 1
run 3
I (A)
0
-100
-200
-100
300
E (mV)


B)
run 1
run 3
I (A)
0
-100
-200
-100
300
E (mV)

CHAPTER 7
CONCLUSIONS AND FUTURE WORK

It has been shown that surfactant modification of graphite surfaces improves the response of inactive surfaces such as GC and improves the stability of active but easily fouled RPG. The results indicate that ionic surfactants interact head-on at GC and RPG through electrostatic interactions with the hydrophilic, negatively charged surface groups driving the assembly. Therefore, cationic surfactants interact more favorably with these surfaces. The results indicate that electrostatic interactions at GC are more important than at RPG despite the greater negative charge density of RPG. At RPG a combination of electrostatic and hydrophobic interactions is important to good response. Because of differences in surface structure (e.g. heterogeneity (roughness)), the microstructure of surfactant assemblies differs at GC and RPG, where surfactant assembly at GC is thought to be more bilayer-like and at RPG more micelle-like. Due to the different probe-surface interactions at RPG and GC, surfactants effect on probe response differs at these surfaces. An enhancement in catecholamine response is observed at inactive GC and a reduction in adsorption of catecholamines, leading to a reduction in surface changes, is observed at RPG.

The nature of the double layer composition had a more significant effect on catecholamine response at the bare surface than at the surfactant-modified surface. Again these effects were more apparent at GC where electrostatic interactions are important. Effective charge screening observed at high ionic strength or in the presence of multiply charged cations results in an improvement in response for anionic species and a reduction in response for cationic ones at GC. Similarly, anionic surfactant assembly at GC was somewhat improved with effective charge screening. At RPG, catecholamine response was best at high ionic strength, when other interactions with the surface such as hydrophobic ones were optimum and surfactant assembly was insensitive to changes in the double layer composition. Most importantly, these results showed that surfactants provide a favorable amphophilic environment for catecholamine response which can not be achieved simply by controlling double layer composition.

Application of biological surfactants, bile, in the modification of GC and RPG for the electrochemical analysis of a redox protein proved to be successful in providing a sensitive and stable response while maintaining the protein in native form. In addition, the results provided a model for bile-protein interactions which provides insight into the parameters needed for good response of large as well as small biological molecules. From these results it can be concluded that for good response of a redox protein, weak (electrostatic or hydrogen bonding) modifier-protein interactions are best. These interactions leave the tertiary structure of the protein intact. In addition to weak protein-modifier interactions, modifier-surface interactions must be favored over protein-surface interactions in order to prevent direct protein-surface interactions, since this can lead to permanent protein adsorption at the surface and eventual loss of signal.

Bile surfactants proved to be such a modifier since bile-protein interactions are electrostatic, as confirmed by the measured $E^{\circ\prime}$. In addition, the improvement in surface stability and sensitivity observed at both surfaces in bile solutions indicates that bile assembly at the surface is favored over that of the protein Finally these results confirm that the assembly of bile surfactants at GC and RPG is driven by hydrophobic interactions since favorable electrostatic interactions between positively charged protein and catecholamine were observed

Ionic surfactant modification of carbon fiber electrodes also proved to be successful in the analysis of catecholamines Because of the high density of edge planes reported at this surface cation preconcentration is observed at low ionic strength when these negative surface groups are most exposed. This is consistent with the higher limiting currents observed for positively charged catecholamines than negatively charged ones However at high ionic strength when these negative sites are screened other interactions with the surface such as hydrophobic ones exist. These interactions result in similar catecholamine-surface interactions for both negative and positive charged probes

Ionic aliphatic surfactant assembly at carbon fiber electrodes appears to be driven by electrostatic effects since surfactant assembly is significantly affected by ionic strength Similarly to what was observed for catecholamines at conventional size electrodes anionic aliphatic surfactants (SDS) assembled best at high ionic strength and cationic ones (CTAB) were assembled best at low ionic strength Again, cationic surfactant assembly was more effective than anionic surfactant assembly consistent with the results at GC and RPG Furthermore, ionic aliphatic surfactant modification of carbon fiber disk electrodes was effective in reducing surface changes This was apparent from the increase in stability of response of catecholamines observed in the presence of surfactants Surface changes due to surface oxidation were shown to be reduced at faster scan rates consistent with previous work (2) The combination of faster scan rates with surfactant modification proved to be best for sensitive and stable catecholamine response

Future work in this area should be aimed at applying these modified surfaces as electrochemical detectors for chromatographic methods such as HPLC and CZE. Surfactants in liquid chromatography have been shown to improve separation efficiency, reduce band broadening and improve resolution (1, 106-109) Surfactants act as a pseudostationary phase in separations Improving resolution for structurally similar species as a result of differential partitioning of the analytes within the interior of the micelles where more hydrophobic compounds partition deeper than less hydrophobic ones (110) Surfactants reduce adsorption of analyte on a reverse phase stationary phase thus decreasing their retention times This also results in a reduction in peak broadening due to a reduction of analyte adsorption on the column (106) In addition the need for organic solvents which can cause protein denaturing can be avoided in surfactant media (106) Therefore, with proper choice of surfactant, both the separation and the electrochemical detection of biological molecules can be easily improved

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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